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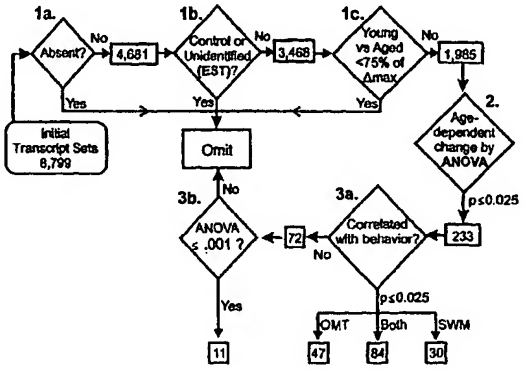
(54) Title: GENE EXPRESSION PROFILE BIOMARKERS AND THERAPEUTIC TARGETS FOR BRAIN AGING AND AGE-RELATED COGNITIVE IMPAIRMENT



(57) Abstract: A statistical and functional correlation strategy to identify changes in cellular pathways specifically linked to impaired cognitive function with aging. Analyses using the strategy identified multiple groups of genes expressed in the hippocampi of mammals, where the genes were expressed at different levels for several ages. The aging changes in expression began before mid-life. Many of the genes were involved in specific neuronal and glial pathways with previously unrecognized relationships to aging and/or cognitive decline. The processes identified by the strategy suggest a new hypothesis of brain aging in which initially decreased neuronal activity and/or oxidative metabolism trigger separate but parallel genomic cascades in neurons and glia. In neurons, the cascade results in elevations in calcium signaling and reductions of immediate early gene signaling, biosynthesis, synaptogenesis and neurite remodeling. In contrast, glia undergo increased lipid metabolism and mediate a cycle of demyelination and remyelination that induces antigen presentation, inflammation, oxidative stress and extracellular restructuring. These identified genes and the proteins they encode can be used as novel biomarkers of brain aging and as targets for developing treatment methods against age-related cognitive decline, Alzheimer's Disease and Parkingson's Disease.

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PCT/US 96/ 14839

INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Please see Further Information sheet enclosed.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Remark : Although claims 65-83 could be considered as non searchable (Rule 39.1 (iv) PCT) the search has been carried out as far as possible.

INTERNATIONAL SEARCH REPORT

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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9210588	25-06-92	AU-A-	1248292	08-07-92
		EP-A-	0562047	29-09-93
WO-A-9511995	04-05-95	AU-A-	8126694	22-05-95
		EP-A-	0730663	11-09-96
WO-A-9500530	05-01-95	AU-A-	7212494	17-01-95
		EP-A-	0705271	10-04-96
US-A-5202231	13-04-93	US-A-	5492806	20-02-96
		US-A-	5525464	11-06-96
WO-A-9015070	13-12-90	US-A-	5143854	01-09-92
		AT-T-	110738	15-09-94
		AU-B-	651795	04-08-94
		AU-A-	5837190	07-01-91
		AU-B-	672723	10-10-96
		AU-A-	7765594	04-05-95
		CA-A-	2054706	08-12-90
		DE-D-	69012119	06-10-94
		DE-T-	69012119	22-12-94
		EP-A-	0476014	25-03-92
		EP-A-	0619321	12-10-94
		ES-T-	2058921	01-11-94
		GB-A,B	2248840	22-04-92
		HK-A-	61395	05-05-95
		HK-A-	64195	05-05-95
		IL-A-	94551	30-03-95
		JP-T-	4505763	08-10-92
		NL-B-	191992	01-08-96
		NL-T-	9022056	02-03-92
		SG-A-	13595	16-06-95
		US-A-	5489678	06-02-96
		US-A-	5547839	20-08-96
		US-A-	5424186	13-06-95
		US-A-	5405783	11-04-95
		US-A-	5510270	23-04-96
		US-A-	5445934	29-08-95
		US-A-	5527681	18-06-96

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 96/14839

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9004652	03-05-90	US-A-	5002867	26-03-91
		EP-A-	0439550	07-08-91
		JP-T-	4501362	12-03-92
WO-A-8910977	16-11-89	AT-T-	110790	15-09-94
		DE-D-	68917879	06-10-94
		DE-T-	68917879	05-01-95
		EP-A-	0373203	20-06-90
		JP-T-	3505157	14-11-91
EP-A-0717113	19-06-96	NONE		
EP-A-0721016	10-07-96	US-A-	5556752	17-09-96

GENE EXPRESSION PROFILE BIOMARKERS AND THERAPEUTIC TARGETS FOR BRAIN AGING AND AGE-RELATED COGNITIVE IMPAIRMENT

STATEMENT OF GOVERNMENT INTEREST

[01] This invention has been made in part with government support under grants AG04542, AG10836, AG18228 and AG14979 from the National Institute on Aging, and by MH59891. The government of the United States of America may have certain rights in this invention.

FIELD OF THE INVENTION

[02] The invention relates generally to genetic algorithms, and more particularly to the identification of gene expression profile biomarkers and therapeutic targets for brain aging.

BACKGROUND OF THE INVENTION

[03] Brain aging processes are enormously complex phenomena that affect multiple systems, cell types and pathways, and result in cognitive decline and increased risk of Alzheimer's disease (AD). Landfield PW *et al.*, *J Neurobiol 23*: 1247-1260 (1992). Although several biological mechanisms have been putatively linked to brain aging or Alzheimer's disease, including inflammation, oxidative stress, Ca^{2+} dyshomeostasis (Landfield, PW & Pitler TA, *Science 226*: 1089-1092 (1984); Landfield PW *et al.*, *J Neurobiol 23*: 1247-1260 (1992)), mitochondrial dysfunction and chronic exposure to adrenal stress hormones (Landfield PW *et al.*, *Science 214*: 581-584 (1981); Porter NM & Landfield PW, *Nature Neurosci 1*: 3-4 (1998)), the specific mechanisms and pathways, if any, through which they are linked to impaired brain function are not understood.

[04] It is widely thought that gene expression changes contribute to many aspects of declining function with aging. Finch CE, *Longevity, Senescence and the Genome,* 37-42 (Univ. Chicago Press, Chicago, 1990). It is also thought that gene expression changes are important for processing and storage of memory. However, not all genes that change expression in the brain with aging are thought to be important for cognition.

[05] Gene-expression changes that specifically contribute to age-related memory decline should selectively change with brain aging and should be correlated specifically with measures of age-associated cognitive decline; that is, a subset of the full set of

aging-dependent genes should also correlate with age-related cognitive decline. *See*, Lockhart DJ & Barlow C, *Nat Rev Neurosci 2:* 63-68 (2001) and Mirnics K, *Nat Rev Neurosci 2:* 444-447 (2001).

[06] If a subset of age-dependent genes also shows expression patterns directly correlated with age-related memory decline, then such a subset of "aging and cognition-related genes" (ACGs) would be extremely helpful as biological indexes ("biomarkers") for assessing or diagnosing the degree of age-related cognitive impairment in individual subjects. In turn, the ability to measure aging-related cognitive impairment quantitatively is essential for discovering new therapeutic targets, and developing new strategies and pharmaceutical compounds for counteracting normal age-related cognitive decline and/or age-related neurodegenerative diseases, including Alzheimer's disease (AD) or Parkinson's disease (PD).

[07] Identifying ACGs in any mammalian species therefore, might have great therapeutic usefulness. Moreover, because of the well-established homologies of most genes across mammalian species and because of the clear similarities in patterns of brain aging and cognitive decline across species, identification in any mammal would have human health implications. Furthermore, because the primary risk factor for Alzheimer's disease and Parkinson's disease is aging itself, therapeutic approaches developed for aging-related cognitive impairment should also help ameliorate cognitive decline from age-related neurodegenerative disease. Thus, there is a clear need for identifying ACGs but, to date, such genes have not been discovered for any mammal.

[08] Gene microarray technology provides a powerful approach for unraveling the complex processes of aging. To date, however, its impact has been limited by statistical problems, small sample sizes, and difficulty in assessing functional relevance. Moreover, studies that have examined gene expression during brain aging using microarrays have not used sample sizes large enough to provide adequate statistical power for formal statistical testing. Lee CK *et al., Nature Genetics 25*: 294-297 (2000); Jiang CH *et al., Proc Natl Acad Sci USA 98*: 1930-1934 (2001) Therefore, even the genes they have reported to change with aging have not been validated by accepted statistical criteria.

[09] The extremely large data sets generated by microarrays pose formidable bioinformatics and resource problems that have to date limited the impact of this powerful technology. Because of these difficulties, most microarray studies have relied on simple fold change comparisons in small samples. However, neither fold change analyses nor the small

sample protocols widely used allow the direct estimates of variance necessary for defining type I error (false positives). In addition, fold change criteria, by definition, select for large changes. Therefore, they exhibit low detection sensitivity (high false negatives, or type II error), and are unable to identify the modest changes that often characterize functionally important (and, therefore, tightly regulated) genes. The inability to assign type I error is a particularly critical problem for microarray studies because the thousands of comparisons of gene expression in such analyses greatly increase the expected false positives. For example, even if group sizes were sufficient for formal statistical analyses, and 5000 gene transcripts were each tested by *t-test* for differences between two conditions at $p \leq 0.05$, the false positive rate is equal to the *p-value* and, consequently, 5% of the 5000 tested transcripts (250) would be expected to be found significant by chance alone.

[10] Although microarray studies have some important offsetting advantages that improve statistical confidence (*e.g.*, co-regulation of genes within a functional group), there is increasing recognition that microarray experiments should generally meet the same statistical standards as other biological experiments or, at least, should systematically estimate the degree of statistical uncertainty. Several strategies to improve statistical confidence have been developed for small-sample microarray studies, but these generally rely on indirect estimates of variance and/or greatly sacrifice sensitivity (*i.e.*, stringent *p-values*).

[11] Another highly important problem of microarray studies is that of determining which of the hundreds of expression changes that may be observed are likely to be functionally relevant. Correlation analysis is one quantitative approach to linking gene expression with function, although it also requires relatively large sets of independent samples. Expression-function correlations fulfill a key prediction of a causal relationship (*i.e.*, that causally related variables should co-vary) and therefore, can serve as a valuable tool for the identification of candidate functionally relevant genes. Nonetheless, there have been few correlation studies attempting to link cognitive dysfunction with univariate gene expression patterns across individual subjects, much less using the massive amounts of data generated in microarray analyses.

SUMMARY OF THE INVENTION

[12] The invention provides a statistical and functional correlation strategy to identify changes in cellular pathways specifically linked to impaired cognitive function with aging.

The bioinformatics and functional correlation strategy improves the power of microarray analyses and provides the ability to test whether alterations in specific hippocampal pathways are correlated with aging-related cognitive impairment. The invention is useful for application in large, well-powered groups and for controlling type I error (false positives), enhancing detection sensitivity (reducing type II false negatives) and determining which aging changes in expression are most closely correlated with declining brain function.

[13] Accordingly, the invention provides a method for identifying a biomarker for brain aging, where the biomarker is a polynucleotide or a polypeptide encoded by said polynucleotide. The method involves first obtaining a set of polynucleotides obtained from a set of brain samples (such as hippocampal samples), where the members of the set of brain samples were obtained from members of a set of mammals, wherein the set of mammals contains more than two members, with at least young, mid-aged and aged members, and then identifying the identity and amount of the members of the set of polynucleotides present in the brain samples. The method then involves the steps of deleting certain non-biomarker polynucleotides from the set of polynucleotides, testing by a conventional statistical method (such as) for a significant effect of aging across the young, mid-aged and aged members; and correlating the identity and amount of the members of the set of polynucleotides present in the brain samples with cognitive performance in behavioral tests.

[14] By use of the methods of the invention, one skilled in the genomics art can identify multiple groups of related genes, many representing processes with previously unrecognized relationships to aging and/or cognitive dysfunction. Thus, the invention also provides compositions of matter comprising sets of genes, expressed sequence tags (ESTs), polynucleotides and polypeptides encoded by said polynucleotides identified as being involved in the aging processes. These sets usefully result in a statistically validated, comprehensive overview of mammalian, including human, functional brain aging. In particular, the set of genes can be used for the diagnosis of human age-related disease, such as an age-related neurodegenerative condition, including Alzheimer's disease or Parkinson disease.

[15] The invention provides a set of biomarkers for brain aging, where (a) the set of biomarkers comprises at least two members; (b) the brain expression patterns of the members of the set are significantly altered with aging as determined by a conventional statistical method (such as ANOVA or student's t test), with $p < 0.05$; (c) the brain expression patterns

of the members of the set are correlated (using a conventional statistical correlation test, *e.g.*, tested by Pearson's or Spearman's correlation test) across age groups with cognitive performance in behavioral tests, with a correlation of $p < 0.05$ (or with a more stringent correlation of $p < 0.01$ or $p < 0.001$) between brain expression and cognitive performance; and (d) the cognitive performance in behavioral tests significantly altered with aging as determined by a conventional statistical method. The biomarkers may also correlate with a behavioral measure of functional impairment, such as an age-related neurodegenerative condition, including Alzheimer's disease or Parkinson's disease.

[16] The invention also provides a set of at least two biomarkers for brain aging, where where the brain expression patterns of the members of the set are significantly altered with aging as measured by a conventional statistical correlation test at a significance level of $p < 0.01$.

[17] The invention further provides a set of at least two biomarkers for brain aging, where the brain expression patterns of the members of the set are significantly altered with aging as determined by a conventional statistical method, with $p < 0.05$ (or a more stringent correlation, such as $p < 0.025$, $p < 0.01$ or $p < 0.001$).

[18] In one example of the invention, rats in three age groups (Young, Mid-Aged, Aged) were characterized on two memory tasks and each mammal's hippocampal CA1 region was analyzed by a microarray analysis for gene expression. These analyses identified multiple groups of genes, many representing pathways with previously unrecognized relationships to aging and/or cognitive decline. The analysis showed that for all groups, the aging changes in expression began by mid-life.

[19] In one aspect of the invention, the known interactions of the identified processes suggest an integrative model of specific cellular cascades that begin in mid-life and eventually impair cognitive function and increase neuronal vulnerability. Initially decreased neuronal activity and/or oxidative metabolism trigger separate but parallel genomic cascades in neurons and glia. In neurons, the cascade results in reductions of immediate early gene signaling, biosynthesis, synaptogenesis and neurite remodeling. In contrast, glia undergo increased lipid metabolism and mediate a cycle of demyelination and remyelination that induces antigen presentation, inflammation, oxidative stress and extracellular restructuring. Intervention studies based on these findings can identify the cause and effect interactions among the complex processes of brain aging.

BRIEF DESCRIPTION OF THE DRAWINGS

[20] FIG. 1 is a set of bar graphs showing age-dependent impairment of memory performance. Male Fischer 344 rats aged 4 months (Young, n = 10), 13 months (Mid-Aged, n = 10) and 24 months (Aged, n = 10) were used. Aged animals exhibited significantly reduced performance on 24 hr memory retention on both the Morris spatial water maze task (SWM; FIG. 1A) and object memory task (OMT; FIG. 1B) in comparison to either Young or Mid-Aged animals (*p<.05, **p<.01, by 1-way ANOVA and Tukey's *post-hoc*). As shown in the bar graph, the Young and Mid-Aged animals did not differ significantly from each on either the SWM or OMT task. On the SWM task (FIG. 1A), higher platform crossings reflects greater retention of the spot where the platform was previously located. For the OMT (FIG. 1B), a higher memory index reflects greater retention of the previously explored object, and resultant increased exploration of the novel object.

[21] FIG. 2 is a flow chart for a filtering and statistical test algorithm for identifying primary set of ACGs. The flow chart also includes the results for an example of the invention. An initial set of 8,799 transcript sets contained on the U34A Gene Chip (*see*, EXAMPLE 2) was filtered prior to statistical testing, to reduce expected false positives. Probe sets were removed if they were called "absent" (1a.), if they were unknown expressed sequence tags (ESTs) (1b.) or if the difference between the Young and Aged groups did not comprise at least 75% of the maximal normalized age differences (1c.). Each of the remaining 1,985 transcript (gene) sets was then tested by ANOVA across the three age groups (n=9-10) to determine if it changed significantly with aging (2.). Each of the 233 genes that changed significantly with age ($p \leq 0.025$) was then tested across all animals (n=29) for significant behavioral correlation with OMT, SWM, or both SWM and OMT (Pearson's; 3a). Furthermore, of the genes that did not correlate with behavior, ones that showed an ANOVA p value $\leq .001$ were also retained for further analysis (3b). In total, 172 genes were considered, 161 of which could be considered ACGs.

[22] FIG. 3 is a set of line graphs showing correlation of gene expression and OMT across individual animals. Behavioral correlation is measured across all age groups. For genes that decreased with aging, the five best positive correlations (A) and for genes that increased with aging, the five best negative correlations (B) are shown (see Legend: correlation p-values in parentheses). Standardized values for both expression and OMT performance are shown on

the Y-axis. The animals were ranked for OMT performance on the X-axis, from worst (1) to best (29), and OMT performance was plotted as a heavy black line on both A and B for the purposes of comparison. Genes involved in early responses and synaptic remodeling were among the five most highly correlated genes that decreased with aging, whereas those related to actin assembly and inflammation were among the five most highly correlated genes that increased with aging.

[23] FIG. 4 is a line graph and pie chart insert showing functional categories and age course of genes decreased with aging. Chronological patterns are shown for aging changes for five of the eight functional categories (some categories were omitted to improve legibility and because they were highly similar to the ones already depicted). Each gene's expression was normalized prior to calculating category mean values. Note that most downregulated categories exhibited ≥50% of mean changes by the Mid-Aged point, and showed relatively less change between the Mid-Aged and Aged animals. No category showed a predominantly Mid-to-Aged pattern of change. The pie-chart insert shows proportion of genes that followed each of the three possible routes to decreased expression with aging.

[24] FIG. 5 is a line graph and pie chart insert showing functional categories and age course of genes increased with aging. Chronological patterns are shown for aging changes for five of the eleven functional categories of behaviorally-correlated upregulated genes (some categories were omitted to increase legibility and because their pattern of change with age was highly similar to that of categories already depicted). Calculations and nomenclature as in FIG. 4. Note that, in contrast to the majority of downregulated genes (FIG. 4), changes in upregulated categories did not tend to level off after mid-life but instead showed continuing change between mid-life and late-life (*e.g.*, a monotonic pattern). Similar patterns were seen when all upregulated genes are considered (Pie-chart inset).

[25] FIG. 6 is a micrograph showing a model of parallel neuronal and glial cascades leading to functional impairment. Early in mid-life, initiating factors (*e.g.*, reduced neuronal activity, onset of late-acting gene expression) induce downregulation of neuronal (N) oxidative phosphorylation triggering a cascade of impaired IEG signalling, biosynthetic potential, and critically, decreased capacity for neurite remodeling and synaptogenesis. In parallel, enhanced lipid metabolism and demyelination are triggered in oligodendrocytes (O) by altered energy metabolism or neural activity. In turn, astrocytes (A) hypertrophy and increase glycolysis of the glucose taken up by astrocytic endfeet on capillaries (C).

Simultaneously, phagocytosis of myelin fragments triggers oxidative damage and inflammatory responses in microglia (M). Eventually, the combined effects of reduced synaptic remodeling, decreased activity and axon conduction, altered extracellular matrix and expanding inflammation result in cognitive failure and neuronal vulnerability.

DETAILED DESCRIPTION OF THE INVENTION

[26] The concept of "biomarker" is well-known and useful concept for those of skill in the genomic art. In general, a biomarker is a measurable biological manifestation that is a quantitative indication of the presence and degree of an underlying biological process of interest.

[27] We have devised a multi-stage method for the identification of biomarkers for brain aging, using gene expression microarrays and behavioral testing. The method of the invention allows one skilled in the genomics art to identify both "aging and cognition-related genes" (ACGs) and unique genes that change with brain aging alone, based on formal statistical testing.

[28] As used in this specification, the word "cognitive" is defined as comprising the higher order intellectual/brain processes involved in learning, including attention, acquisition, short-term memory, long-term memory and memory retrieval, among others.

[29] As used in this specification, across different mammalian species, age definitions are as follows: "Young" mammals are those at or beyond reproductive maturity for the species. "Mid-aged" is defined in two ways: at or around half the average lifespan for the species and at or around the midpoint between reproductive maturity and average lifespan. "Aged" mammals are those at or around average lifespan. Animals intermediate between two ages could be considered as part of the group to which they are most closely chronologically related (with the exception of young animals, for whom it would be inappropriate to include prepubescent individuals)

[30] We used the bioinformatics and functional correlation strategy of the invention for microarray analyses. As a result, we were able to detect multiple groups of related genes that were altered by brain aging and also correlated with cognitive function across individual subjects. Most of the shifts in genomic regulation began by mid-life, well before the onset of measurable cognitive impairment, implying that cognitive function is not altered substantially

without further progression and/or the cumulative effects of the initial changes in gene regulation.

[31] This analysis depended on a novel combination of three approaches for microarray research: (a) the quantitative measurement of the dependent function of interest (cognitive performance), which provided a basis for large-scale expression-function correlation analyses; (b) the application of formal statistical analyses (ANOVA, Pearson's) to large groups of independent microarray samples, which conferred substantial statistical power and high detection sensitivity for even modest changes (low false negative type II error); and c) systematic estimates of the maximum probabilities of false positives in our data. Our results using the method of the invention provide a generally comprehensive overview of hippocampal genes/processes that are altered with brain aging and closely linked to brain functional decline.

[32] To verify the method of the invention, we first tested young (3-4 months old), mid-aged (12-13 months old) and aged (24-25 months old) rats (n = 9-10 per group) for performance on the Morris spatial water maze (SWM) and object memory task (OMT). Both behavioral tests clearly and reliably (statistically) revealed aging-related cognitive impairment (FIG. 1).

[33] We then anesthetized (for euthanasia) all animals and dissected out a region of the brain (CA1 region of the hippocampus) known to be important for memory. These brain tissues were then prepared for analyses of gene expression profiles (mRNA content) on Affymetrix GeneChip microarrays specific for the rat genome (RG-U34A arrays) (one array for each individual rat sample). The microarrays were then read and analyzed for expression profile data on an Affymetrix GeneChip System according to the manufacturer's instructions.

[34] The behavioral and microarray methods that were used can reasonably be expected to apply as well to mice as to rats. Similar behavioral and microarray methods known to those of skill in the art can be used for testing of other mammals, including humans. The utility of the method of the invention for human testing is discussed below.

[35] We then transferred the data into standard computer spreadsheets (*e.g.*, Excel) for performing statistical analyses of the effects of aging. Using Analysis of Variance (ANOVA) we defined the set of genes whose degree of expression changed significantly with brain aging. We then used that set of "Aging Genes" and tested each gene's expression profile (across only the aged animals) for significant correlation with memory performance on the

Object Memory Task (OMT) as well as the Morris spatial water maze (SWM). The "Aging Genes" whose expression patterns correlated significantly with cognitive performance were defined as the primary subset of "Aging and Cognition-Related Genes" (ACGs), and subcategorized as OMT-associated, SWM-associated, or both OMT and SWM-associated. We further included genes with no behavioral association that had an ANOVA p value ≤ .001 since genes identified at this more stringent level are less subject to the error of multiple testing (FIG. 2, TABLES 1A and 1B).

[36] Based on those large-scale studies, we have developed a list of ACGs that appear to have considerable potential importance for assessing and generating new treatments for age-dependent functional decline (TABLES 1A and 1B).

[37] These lists contain some genes that were identified previously as being linked to brain aging or neurodegeneration (*e.g.*, inflammation or mitochondrial genes, Lee CK *et al., Nature Genetics 25*: 294-297 (2000)) but none has been previously shown to be specifically associated with both brain aging and aging-dependent cognitive impairment. Further, many genes on our list have not even been shown previously to be linked to brain aging alone or to cognition alone. Thus, our lists of ACGs are unique and useful biomarkers and therapeutic targets specifically for aging-dependent cognitive impairment. In addition, our list of all genes that change with brain aging contains many genes never before reported to change with brain aging, and therefore provides a useful and unique panel of gene biomarkers and therapeutic targets for study and treatment of brain aging.

[38] In addition to these lists for identified genes, we have also performed the same analyses and compiled the same lists for unidentified expressed sequence tags (ESTs) that are on the same Affymetrix Chips (TABLE 2). These are valuable data, because once the ESTs are identified, they can provide therapeutic targets.

[39] Using the method of the invention, we were able to identify a number of processes and pathways that previously have not been clearly associated with normal brain aging. The most unexpected findings included altered expression profiles suggestive of increased myelin and lipid turnover, as well as widespread changes indicating coordinated downregulation of oxidative metabolism, decreased neurite outgrowth and synaptogenesis. Other novel genes we identified appear to suggest alterations in general metabolic and biosynthetic chaperone functions. In addition, many of the identified groups confirmed previously described changes in expression for genes regulating several major processes (*e.g.*, inflammation, glial

reactivity, oxidative stress). However, our results also extend the earlier findings considerably by revealing the extent of the changes and the concurrent upregulation of potentially orchestrating transcription factors and cytokines that may provide important clues to pathogenic mechanisms.

[40] In order to begin to develop an integrative overview of potential interactions among the multiple altered expression patterns observed here, we considered functional implications at the pathway level. Our interpretations rely on the functions that have been previously associated with many of the genes identified by those of skill in the genomics art. These are identified through PubMed literature searches, annotations provided by Affymetrix, entries in the SwissProtein database <http://www.expasy.ch/sprot/sprot-top.html> and associations reported in the Genome Ontology (GO; <http://www.geneontology.org>). We also rely on the general assumption held by those of skill in the genomics art that similar changes in the expression of multiple genes of a particular pathway imply like changes in the functions mediated by the encoded proteins of that pathway. Gene expression changes also can reflect compensatory negative feedback regulation (or other dissociations of gene expression and protein function), but the potential confound of dissociation is presumably less of a problem in microarray analyses in which multiple genes in a pathway are observed to change in the same direction. Some of the primary metabolic pathways and processes considered in the interpretations are depicted in TABLE 1.

[41] *Functional Groups.* We found age-dependent upregulation of many ACGs involved in inflammatory/immune/stress responses and downregulation of many involved in energy metabolism. In addition, we found alterations of gene expression reflecting multiple categories/pathways not previously recognized to change with normal aging. These included upregulation of genes for myelin proteins, cholesterol biosynthesis and transport, amino acid metabolism, intracellular Ca^{2+} signaling, and protein processing, as strongly suggesting an ongoing cycle of remyelination and demyelination. We also found widespread downregulation of genes for biosynthesis, immediate early responses, and synaptic structural plasticity, suggestive of neuronal involution. Multiple transcriptional regulators and cytokines were also identified that may play orchestrating roles. Nearly all expression changes began by mid-life but cognition was not impaired until late life. Upregulated genes for inflammation and intracellular Ca^{2+} release were among those most closely correlated with impairment.

TABLE 1A

Functionally Grouped ACGs and Genes Showing Highly Significant Age-Dependent Decreases in Expression

GenBank	Description	Young	Mid	Age	ANOVA p	beh all
Synaptic Structural Plasticity						
M64780*	Agrn, Agrin	2746 ± 105	2334 ± 74	2207 ± 79	0.0005	Both
L21192	GAP-43, membrane attached signal protein 2 (brain)	10324 ± 546	8990 ± 327	8165 ± 480	0.0095	Both
S82649	Narp, neuronal activity-regulated pentraxin	4358 ± 300	3470 ± 143	3247 ± 185	0.0029	OMT
M74223	VGF, neurosecretory protein	6697 ± 373	5836 ± 387	4722 ± 369	0.0042	OMT
U63740*	Fez1, Protein kinase C-binding protein Zeta1	10339 ± 180	9322 ± 258	9388 ± 330	0.0239	OMT
AB003726	Homer1a, RuvB-like protein 1	3546 ± 270	2354 ± 121	2469 ± 132	0.0001	None
U19866	Arc, activity-regulated cytoskeleton-associated protein	6374 ± 527	4408 ± 228	4094 ± 398	0.0008	None
Transcription Regulator						
M18416	Egr1, Early growth response 1 (Krox-24)	4911 ± 259	3688 ± 177	3544 ± 165	0.0001	Both
M92433	NGFI-C, Zinc-finger transcription factor	2037 ± 149	1576 ± 44	1495 ± 70	0.0009	Both
L08595	Nuclear receptor subfamily 4, group A, member 2	1467 ± 80	1186 ± 83	1011 ± 62	0.0010	Both
AI030089	Nopp130, nucleolar phosphoprotein p130	471 ± 31	397 ± 31	314 ± 22	0.0022	Both
AF016387	RXRG, retinoid X-receptor gamma	1900 ± 129	1503 ± 95	1365 ± 103	0.0059	Both
AA800794	HT2A, zinc-finger protein	2480 ± 67	2396 ± 41	2097 ± 73	0.0004	OMT
AA799641	S164, Contains a PWI domain associated with RNA splicing	7645 ± 169	7690 ± 183	6842 ± 250	0.0106	OMT
U78102	Egr2, Early growth response 2	576 ± 95	223 ± 21	205 ± 23	0.0001	SWM
U44948	SmLIM, smooth muscle cell LIM protein	1166 ± 15	928 ± 55	887 ± 38	0.0001	SWM
AA891717	USF-1, upstream stimulatory factor 1	3607 ± 142	2993 ± 91	3025 ± 66	0.0003	None
AF095576	Aps, adaptor protein with pleckstrin and src homology	526 ± 40	275 ± 49	272 ± 46	0.0007	None
Intracellular Signal Transduction						
AI176689	MAPKK 6, mitogen-activated protein kinase kinase 6	2012 ± 84	1781 ± 92	1528 ± 88	0.0030	Both
X89703	TPCR19, Testis Polymerase Chain Reaction product 19	361 ± 25	320 ± 25	252 ± 24	0.0155	Both
L04485	MAPPK1, mitogen-activated protein kinase kinase 1	13110 ± 365	11951 ± 312	11200 ± 506	0.0104	OMT
AA817892	Gnb2, Guanine nucleotide binding protein (beta 2 subunit)	6500 ± 159	5606 ± 214	5765 ± 218	0.0110	OMT
AF000901	P58/P45, Nucleoporin p58	597 ± 43	444 ± 51	391 ± 47	0.0150	OMT
M87854	Beta-ARK-1, beta adrenergic receptor kinase 1	1994 ± 110	1723 ± 90	1544 ± 114	0.0202	OMT
AF058795	Gb2, GABA-B receptor	9443 ± 360	9064 ± 478	7857 ± 323	0.0228	OMT
AA800517	VAP1, vesicle associated protein	637 ± 72	674 ± 61	455 ± 35	0.0228	OMT
Signal Transduction						
AF003904	CRH-binding protein	773 ± 51	782 ± 35	630 ± 23	0.0119	Both
M15191	Tac1, Tachykinin	1415 ± 110	1078 ± 57	1068 ± 74	0.0093	OMT
AF091563	Olfactory receptor	440 ± 21	367 ± 29	332 ± 27	0.0233	SWM
M64376	Olfactory protein	810 ± 26	605 ± 83	568 ± 57	0.0247	SWM
M15880	Npy, Neuropeptide Y	4647 ± 158	3561 ± 223	3668 ± 141	0.0004	None
Adhesion, Extracellular Matrix						
M27207	Col1a1, Procollagen- type I (alpha 1)	678 ± 24	521 ± 43	480 ± 23	0.0005	Both
AF104362	Omd, Osteomodulin (osteoadherin)	289 ± 16	217 ± 24	185 ± 15	0.0024	Both
D63886	MMP16, matrix metalloproteinase 16	664 ± 23	604 ± 37	542 ± 19	0.0180	Both
M21354	Col3a1, collagen type III alpha-1	203 ± 22	157 ± 13	132 ± 9	0.0120	SWM
AB010437	CDH8, Cadherin-8	163 ± 24	100 ± 12	83 ± 17	0.0128	SWM

TABLE 1A
Functionally Grouped ACGs and Genes Showing
Highly Significant Age-Dependent Decreases in Expression

GenBank	Description	Young	Mid	Aged	ANOVA p	beh all
Metabolism						
L03294	Lpl, lipoprotein lipase	1147 ± 69	918 ± 40	749 ± 37	0.0000	Both
S68245	Ca4, carbonic anhydrase 4	2272 ± 75	1993 ± 63	1825 ± 54	0.0002	Both
AA859975	LOC64201, 2-oxoglutarate carrier	4792 ± 68	4370 ± 102	4255 ± 97	0.0010	Both
M24542	RISP, Rieske iron-sulfur protein	10337 ± 308	9095 ± 327	8833 ± 128	0.0013	Both
M18467	Got2, glutamate oxaloacetate transaminase 2	9470 ± 241	8355 ± 179	8332 ± 322	0.0061	Both
X64401	Cyp3a3, Cytochrome P450- subfamily IIIA (polypeptide 3)	805 ± 64	762 ± 51	581 ± 34	0.0089	Both
U83880	glycerol-3-phosphate dehydrogenase, mitochondrial	2054 ± 73	1988 ± 77	1673 ± 111	0.0127	Both
J05499	GLS, glutaminase (mitochondrial)	915 ± 24	844 ± 44	787 ± 14	0.0238	Both
U90887	Arg2, arginase type II	499 ± 21	374 ± 31	364 ± 22	0.0015	OMT
M22756	Ndufv2, mitochondrial NADH dehydrogenase (24 kDa)	12293 ± 574	10193 ± 670	9260 ± 750	0.0134	SWM
Transporters, Carriers						
L46873	Slc15a1, Oligopeptide transporter	426 ± 30	411 ± 24	292 ± 27	0.0028	Both
AB000280	PHT1, peptide/histidine transporter	802 ± 20	659 ± 40	691 ± 37	0.0198	OMT
U87627	MCT3, putative monocarboxylate transporter	687 ± 33	521 ± 22	480 ± 38	0.0002	SWM
AA799389	Rab3B, ras-related protein	353 ± 21	324 ± 25	251 ± 23	0.0150	SWM
Growth, Biosynthesis, Maintenance						
X16554	Prps1, Phosphoribosyl pyrophosphate synthetase 1	3159 ± 81	2747 ± 74	2637 ± 97	0.0006	Both
U66470	rCGR11, Cell growth regulator	820 ± 31	676 ± 31	662 ± 38	0.0051	Both
M37584	H2AZ, H2A histone family (member Z)	5335 ± 73	4906 ± 186	4600 ± 162	0.0090	Both
U90610	Cxcr4, CXC chemokine receptor	811 ± 56	812 ± 59	614 ± 29	0.0109	Both
AA874794	Bex3, brain expressed X-linked 3	16735 ± 376	14986 ± 588	14238 ± 457	0.0047	OMT
AA892506	coronin, actin binding protein 1A	4101 ± 121	3625 ± 114	3558 ± 135	0.0104	OMT
AA893939*	DSS1, deleted in split hand/ split foot protein 1	4201 ± 76	3860 ± 129	3658 ± 141	0.0149	OMT
AF087037	Btg3, B-cell translocation gene 3	652 ± 55	676 ± 71	460 ± 29	0.0163	OMT
U06099	Prdx2, Peroxiredoxin 2	12667 ± 675	11742 ± 641	10339 ± 272	0.0216	OMT
AI172476	Tieg-1, TGF-beta-inducible early growth response protein 1	1127 ± 99	925 ± 63	812 ± 53	0.0177	SWM
AA866411	Necdin, neuronal growth suppressor	1994 ± 81	1568 ± 86	1542 ± 62	0.0005	None
Protein Processing and Trafficking						
X54793	Hsp60, heat shock protein 60	10088 ± 333	9602 ± 299	8693 ± 229	0.0071	Both
AA875047	TCPZ, T-complex protein 1 (zeta subunit)	997 ± 161	728 ± 99	470 ± 59	0.0095	Both
D21799	Psmb2, Proteasome subunit (beta type 2)	7298 ± 242	6892 ± 229	6395 ± 177	0.0241	Both
U53922	Hsj2, DnaJ-like protein (RDJ1)	10716 ± 382	8836 ± 190	8392 ± 204	0.0000	SWM
X78605	rab4b, ras-homologous GTPase	3131 ± 292	2040 ± 196	2006 ± 135	0.0012	None

[42] For TABLE 1A, "GenBank" is the gene accession number established at the web accessible GenBank database <http://www.ncbi.nlm.nih.gov/>, The "Description" includes a 'common name' (if applicable) as well as a brief description of the gene product. Values for Young, Mid-Aged, and Aged categories are the mean ± SEM of expression values. Genes are put into functional categories (*see*, above) and grouped by their level of association with behavior (expression correlated significantly (Pearson's; $p \leq .025$) with both tasks, with the OMT, with the SWM, or with none of the tasks but highly significant across age ($p \leq .001$ on ANOVA across age, $p > .025$ for correlation on both SWM and OMT). Within each level of

association, genes are ranked by the significance of the age-dependent change in their expression level (ANOVA; $p \leq .025$). Asterisked (*) genes are those that also showed a significant behavioral correlation (Pearson; $p \leq .025$).

[43] ACGs that were downregulated with aging (TABLE 1A) appeared primarily to represent metabolic and neuronal functions (FIG. 3a).

[44] *Metabolism*. Multiple genes related to functions of the mitochondrial electron transport chain (*e.g.*, glycerol 3-phosphate dehdrogenase, NADH dehydrogenase, Rieske's iron-sulpher protein) were downregulated with aging (TABLE 1A). Moreover, we found aging-dependent downregulation of several genes related to pathways important for glucogenic amino acid catabolism, including glutaminase and arginase (TABLE 1A).

[45] *Synaptic Structural Plasticity*. One of the most prominent categories of identified genes showing decreased expression and behavioral correlation was that comprising genes involved in synaptic structural plasticity, including neurite outgrowth and synaptogenesis (*e.g.*, decreased expression of genes encoding agrin, GAP-43, Homer 1a, Narp, Arc, *etc.*) (TABLE 1A). Many of these genes are activity-dependent in neurons and have been linked previously to synaptic plasticity, neurite remodeling or learning in univariate studies (*e.g.*, Biewenga JE *et al., Acta Biochim Pol 43:* 327-38 (1996); Steward O *et al., Neuron 21:* 741-51 (1998), Mantych KB & Ferreira A, *J Neurosci 21:* 6802-9 (2001), Guzowski JF *et al., J Neurosci 20:* 3993-4001 (2000), Bezakova G, *et al. Proc Natl Acad Sci U S A 98* 9924-9 (2001)), although Gap-43 is one of the few reported so far to change with aging. Similarly, many other neural activity-dependent genes, including IEGs in the Transcription Regulators and Signaling categories (*e.g.*, Egr1, Egr 2, MAPKK, *etc.*), showed decreased expression with aging and were correlated with impaired cognition (TABLE 1A).

[46] In addition, multiple genes important for general growth and biosynthetic mechanisms, chaperone functions and protein processing were also downregulated with aging (*e.g.*, hsp60, histone H2AZ, proteasome subunit, DNA J-like homolog, *etc.*) as were specific neuronal signaling genes (*e.g.*, GluR 5-2, the kainate receptor; and neuropeptide Y) (TABLE 1A). These widely downregulated biosynthetic and signaling genes appear to reflect a general involution of metabolic and neurite structural remodeling processes in neurons (*e.g.*, FIG. 4, TABLE 1A). Chaperone proteins such as the DNA-J-like homolog and hsp60 play critical roles in preventing protein aggregates (Satyal SH *et al., Proc Natl Acad Sci U S A 97* 5750-5 (2000)), which are known to be critical in Alzheimer's disease (Price DL & Sisodia SS, *Annu*

Rev Neurosci 21: 479-505 (1998), Kovacs DM &Tanzi RE, *Cell Mol Life Sci 54:* 902-9 (1998); Sisodia SS *et al., Am J Hum Genet 65:* 7-12 (1999), Tanzi RE & Parson AB (2000), Selkoe DJ, *Neuron 32:* 177-80 (2001)), and could therefore have implications for age-dependent vulnerability to Alzheimer's disease.

TABLE 1B

ACGs and Genes Showing Highly Significant Age-Dependent Increases in Expression

GenBank	Description	Young	Mid	Aged	ANOVA P	beh all
Inflammation, Defense, Immunity						
J04488	Ptgds, Prostaglandin D synthase	3976 ± 248	6891 ± 350	8365 ± 438	0.0000	Both
X71127	c1qb, complement component 1- q (beta polypeptide)	885 ± 52	1461 ± 85	1895 ± 102	0.0000	Both
J03752	Microsomal GST-1, glutathione S-transferase	368 ± 43	695 ± 60	910 ± 45	0.0000	Both
L40362*	MHC class I RT1.C-type protein	1755 ± 64	2106 ± 82	2501 ± 77	0.0000	Both
U17919	Aif1, allograft inflammatory factor 1	712 ± 29	990 ± 47	1152 ± 67	0.0000	Both
M15562	MHC class II RT1.u-D-alpha chain	608 ± 73	1194 ± 238	2120 ± 173	0.0000	Both
X13044	Cd74, CD74 antigen	-49 ± 44	155 ± 83	603 ± 100	0.0000	Both
M24324	RTS, MHC class I RT1 (RTS) (u haplotype)	3274 ± 175	4599 ± 363	5822 ± 342	0.0000	Both
M32062	Fcgr3, Fc IgG receptor III (low affinity)	347 ± 25	462 ± 32	557 ± 21	0.0000	Both
AJ222813	Il18, interleukin 18	110 ± 33	208 ± 14	261 ± 16	0.0002	Both
L40364	RT1Aw2, RT1 class Ib	2033 ± 126	2546 ± 127	2842 ± 115	0.0004	Both
AI231213	Kangai 1, suppression of tumorigenicity 6	2727 ± 116	2952 ± 120	3484 ± 139	0.0008	Both
AI170268	Ptgfr, Prostaglandin F receptor	6651 ± 248	8057 ± 336	8502 ± 359	0.0013	Both
X52477	C3, Complement component 3	34 ± 49	236 ± 83	476 ± 100	0.0034	Both
X73371	FCGR2, Low affinity immunoglobulin gamma Fc receptor II	218 ± 19	285 ± 24	384 ± 21	0.0001	OMT
X78848	Gsta1, Glutathione-S-transferase (alpha type)	3145 ± 74	3909 ± 188	4155 ± 204	0.0009	OMT
AA818025*	Cd59, CD59 antigen	6465 ± 265	7269 ± 163	7474 ± 189	0.0052	OMT
AA891810	GST, Glutathione S-transferase	1136 ± 83	1411 ± 70	1791 ± 101	0.0001	SWM
U92081	Gp38, Glycoprotein 38	547 ± 26	679 ± 38	802 ± 66	0.0037	SWM
X62322	Grn, Granulin	4514 ± 145	4972 ± 254	5375 ± 119	0.0116	SWM
Transcription Regulator						
X13167*	NF1-A, nuclear factor 1 A	112 ± 30	265 ± 38	300 ± 26	0.0008	Both
U67082	KZF-1, Kruppel associated box (KRAB) zinc finger 1	472 ± 31	565 ± 32	617 ± 29	0.0099	Both
U92564	Roaz, Olf-1/EBF associated Zn finger protein	429 ± 50	687 ± 71	761 ± 50	0.0014	OMT
L16995	ADD1, adipocyte determ./ different.-dependent factor 1	784 ± 100	1054 ± 75	1179 ± 95	0.0160	OMT
AI237535	LitaF, LPS-induced TNF-alpha factor	979 ± 62	1078 ± 68	1338 ± 114	0.0193	OMT
AI177161	Nfe2l2, NF-E2-related factor 2	544 ± 31	590 ± 36	687 ± 25	0.0096	SWM

TABLE 1B

ACGs and Genes Showing Highly Significant Age-Dependent Increases in Expression

GenBank	Description	Young	Mid	Aged	ANOVA P	beh all
Signal Transduction						
U26356	S100A1, S100 protein (alpha chain)	1382 ± 105	1636 ± 76	1999 ± 115	0.0008	Both
AA850219	Anx3, Annexin A3	438 ± 26	501 ± 21	575 ± 26	0.0023	Both
D84477	Rhoa, ras-related homolog A2	749 ± 108	1069 ± 111	1319 ± 85	0.0024	Both
AF048828	VDAC1, voltage-dependent anion channel 1	2334 ± 294	3157 ± 392	3844 ± 290	0.0137	Both
AI102103	Pik4cb, Phosphatidylinositol 4-kinase	975 ± 63	1029 ± 67	1252 ± 80	0.0247	Both
L35921	Ggamma, GTP-binding protein (gamma subunit)	498 ± 30	543 ± 43	712 ± 64	0.0108	SWM
M83561	GluR-5, kainate sensitive glutamate receptor	248 ± 23	359 ± 22	351 ± 12	0.0007	None
Adhesion, Extracellular Matrix						
E13541	Cspg5, chondroitin sulfate proteoglycan 5	3938 ± 342	5112 ± 312	5980 ± 242	0.0003	Both
X83231	PAIHC3, Pre-alpha-inhibitor, heavy chain 3	2586 ± 110	2974 ± 180	3460 ± 183	0.0038	OMT
AF097593	Ca4, cadherin 2- type 1 (neuronal)	615 ± 45	855 ± 61	881 ± 59	0.0049	OMT
Myelin-Related Proteins						
M55534	Cryab, alpha crystallin polypeptide 2	2889 ± 155	4153 ± 196	4621 ± 238	0.0000	Both
D28111	MOBP, myelin-associated oligodendrocytic basic protein	13950 ± 386	15483 ± 633	18407 ± 909	0.0004	Both
X06554	S-MAG, myelin-associated glycoprotein C-term	5282 ± 258	5595 ± 140	6564 ± 326	0.0038	Both
S55427	Pmp, peripheral myelin protein	2458 ± 59	2856 ± 148	3080 ± 129	0.0051	OMT
M22357	MAG, myelin-associated glycoprotein	978 ± 163	1544 ± 190	2455 ± 332	0.0010	SWM
Lipid Metabolism/ Transport						
X54096	Lcat, Lecithin-cholesterol acyltransferase	187 ± 35	298 ± 30	417 ± 38	0.0003	Both
S83279	HSDIV, 17-beta-hydroxysteroid dehydrogenase type IV	630 ± 54	685 ± 91	928 ± 67	0.0182	Both
U37138	Sts, Steroid sulfatase	368 ± 74	521 ± 33	587 ± 35	0.0128	OMT
X55572	Apod, Apolipoprotein D	5875 ± 355	7281 ± 601	8343 ± 595	0.0133	OMT
L07736	Cpt1a, Carnitine palmitoyltransferase 1 alpha (liver)	599 ± 65	677 ± 59	854 ± 59	0.0192	OMT
Amino Acid/ Transmitter Metabolism						
J03481	DHPR, Dihydropteridine reductase	13260 ± 369	16897 ± 528	17432 ± 380	0.0000	Both
Z50144	Kat2, kynurenine aminotransferase II	106 ± 33	183 ± 19	240 ± 24	0.0040	Both
U07971	Transamidinase, mitochondrial	2897 ± 130	3311 ± 186	3644 ± 182	0.0183	OMT
M77694	Fah, fumarylacetoacetate hydrolase	847 ± 36	990 ± 49	1305 ± 98	0.0002	SWM
Cytoskeletal, Vesicle Fusion						
X62952	Vim, vimentin	571 ± 100	998 ± 162	1346 ± 122	0.0016	Both
AA892333	Tuba1, alpha-tubulin	-52 ± 83	117 ± 90	357 ± 79	0.0080	Both
U11760*	Vcp, valosin-containing protein	4314 ± 234	5004 ± 333	5651 ± 278	0.0120	Both
U32498*	RSEC8, rat homolog of yeast sec8	-11 ± 37	270 ± 81	232 ± 82	0.0236	OMT
AF083269*	P41-Arc, actin-related protein complex 1b	406 ± 23	488 ± 49	626 ± 72	0.0249	OMT
AF028784	GFAP, glial fibrillary acidic protein	19860 ± 714	19731 ± 1002	23241 ± 1058	0.0217	SWM
Transporters, Carriers						
M94918	Hbb, beta hemoglobin	6172 ± 737	8698 ± 646	13715 ± 1017	0.0000	Both
U31866	Nclone10	3625 ± 302	5416 ± 561	7407 ± 511	0.0000	Both
D38380	Tf, Transferrin	11990 ± 728	16431 ± 707	19831 ± 1519	0.0001	Both
X56325	Hba1, alpha 1 hemoglobin	14433 ± 611	17259 ± 959	23893 ± 1426	0.0000	OMT
AF008439	Natural resistance-associated macrophage protein 2	69 ± 17	153 ± 19	152 ± 13	0.0018	SWM

TABLE 1B

ACGs and Genes Showing Highly Significant Age-Dependent Increases in Expression

GenBank	Description	Young	Mid	Aged	ANOVA P	beh all
Growth, Biosynthesis, Maintenance						
AA799645	FXYD domain-containing ion transport regulator 1	1680 ± 58	2025 ± 68	2457 ± 129	0.0000	Both
L03201	Ctss, cathepsin S	17087 ± 393	19066 ± 691	22376 ± 875	0.0001	Both
M27905	Rpl21, Ribosomal protein L21	11279 ± 905	13999 ± 389	15557 ± 379	0.0001	Both
AA893493	RPL26, Ribosomal protein L26	18442 ± 688	23043 ± 506	24252 ± 1162	0.0001	Both
X52619	Rpl28, Ribosomal protein L28	13167 ± 323	13231 ± 310	14520 ± 228	0.0034	Both
X14181*	RPL18A, Ribosomal protein L18a	8623 ± 430	10171 ± 389	11025 ± 602	0.0068	Both
M31076	TNF-alpha, Transforming growth factor (alpha)	139 ± 23	241 ± 43	295 ± 35	0.0167	Both
AI171462*	Cd24, CD24 antigen	864 ± 69	1270 ± 86	1304 ± 101	0.0026	OMT
X68283	Rpl29, Ribosomal protein L29	9705 ± 262	9500 ± 300	10807 ± 267	0.0050	OMT
X53504*	RPL12, Ribosomal protein L12	9877 ± 328	11398 ± 367	11719 ± 620	0.0241	OMT
U77829	Gas-5, growth arrest homolog	173 ± 15	228 ± 14	264 ± 20	0.0030	SWM
AI234146	Csrp1, Cysteine rich protein 1	4436 ± 335	4925 ± 207	5451 ± 179	0.0243	SWM
Protein Processing and Trafficking						
M32016	Lamp2, lysosomal-associated membrane protein 2	759 ± 38	906 ± 36	1092 ± 74	0.0008	Both
E01534	Rps15, Ribosomal protein S15	16577 ± 368	17202 ± 429	18363 ± 368	0.0116	OMT
AI028975	AP-1, adaptor protein complex (beta 1)	1077 ± 38	1163 ± 69	1317 ± 49	0.0158	OMT
AI175486	Rps7, Ribosomal protein S7	5820 ± 448	6409 ± 312	7212 ± 208	0.0215	OMT
AF023621	Sort1, sortilin	414 ± 34	813 ± 143	812 ± 109	0.0247	OMT
AI230712	Pace4, Subtilisin - like endoprotease	281 ± 31	447 ± 49	570 ± 56	0.0010	SWM
AA891445*	Skd3, suppressor of K^+ transport defect 3	321 ± 24	440 ± 42	508 ± 37	0.0043	SWM
AF031430	Stx7, Syntaxin 7	794 ± 133	1387 ± 188	1461 ± 122	0.0097	SWM
AA900516	Pdi2, peptidyl arginine deiminase (type II)	57 ± 42	314 ± 62	344 ± 51	0.0015	None

[47] The analyses for TABLE 1B are as described for TABLE 1A.

[48] *Upregulated Genes*. Genes that were upregulated with aging and negatively correlated with behavior fit primarily into categories that appeared to reflect activated glial functions (FIGS. 3B and 5, TABLE 1B). Additionally, among the main unexpected findings was a widespread upregulation in the expression of genes encoding proteins for myelin synthesis and lipid turnover (TABLE 1B).

[49] *Lipid Metabolism*. Multiple genes important for mitochondrial and cytosolic lipid β-oxidation (*e.g.*, carnitine palmitoyltransferase, lecithin-cholesterol acyltransferase, *etc.*; TABLE 1B), the primary pathway for free fatty acid (FFA) catabolism, were upregulated.

[50] *Increased Myelin Synthesis, Cholesterol Biogenesis and Vesicle Transport.* Importantly for identifying the trigger mechanism for elevated lipid catabolism, the expression of many genes encoding myelin-related proteins or myelin-related transcription factors on the microarray was increased with aging (and several also were correlated with cognitive impairment) (TABLE 1B). These observations strongly suggest that a major increase in myelin synthesis programs developed with aging. This interpretation is also supported by the upregulation of multiple genes important in lipogenesis for cholesterol

biosynthesis (Add 1/SREBP1), and the packaging/transport of cholesterol esters and other complex lipids (ApoD, LCAT, *etc.*) (TABLE 1B). Recent studies have shown that stimulation of myelin synthesis programs in oligodendrocytes is associated with induction of genes for both myelin proteins and lipogenic pathways (Nagarajan R *et al., Neuron 30* 355-68 (2001)).

[51] *Cyloskeleton/Vesicles*. Moreover, expression of genes related to actin assembly, transport or fusion of packaged vesicles (actin related complex, rsec8, tubulin, and syntaxin 7) was increased (TABLE 1B). These molecules are associated with vesicle transport and fusion in neurons. In addition, however actin assembly proteins are also known to play a major role in myelin vesicle transport in oligodendrocytes (Madison DL *et al., J Neurochem 72:* 988-98 (1999)). Given the upregulation of myelin programs and the downregulation of synaptic plasticity genes, therefore, the age-dependent upregulation of genes linked to vesicle transport capacity seems more likely to be associated with enhanced myelin transport in oligodendrocytes. Further support for the view that extensive oligodendrocyte activation and/or synthesis occurs in hippocampal aging is provided by the observation that many genes that were upregulated with aging are preferentially expressed in oligodendrocytes (*e.g.*, myelin proteins, FAH, PGD-S, *etc.*) (*e.g.*, Labelle Y *et al., Biochim Biophys Acta 1180:* 250-6 (1993)).

[52] Myelin also is normally degraded to free fatty acids through the endosomal-lysosomal pathway. Consistent with elevation of myelin degradation, we also found increased expression of Cathepsin S and other genes encoding lysosomal enzymes (TABLE 1B). Cathepsin S is particularly important in the processing of antigenic myelin fragments.

[53] *Amino Acids*. In contrast to enzymes for glucogenic amino acids (TABLE 1A), expression was upregulated for multiple genes encoding enzymes related to the metabolism of the ketogenic/glucogenic amino acids, tyrosine, phenylalanine and tryptophan (*e.g.*, DHPR, KAT, FAH, *see,* TABLE 1B). Catabolism of ketogenic amino acids yields either acetoacetate or one of its precursors (*e.g.*, acetyl CoA), which can be used either for energy metabolism or lipogenesis. Upregulation of DHPR, which catalyzes the formation of a critical cofactor (tetrahydrobiopterin) for tyrosine and monoamine synthesis, and concomitant upregulation of MAO-B (TABLE 3), together suggest elevated metabolism of tyrosine and tryptophan via greater monoamine turnover.

[54] *Inflammation/Defense/Immunity.* There was massive upregulation of expression of genes encoding MHC class I antigen presenting molecules, and numerous other inflammatory/immune proteins (TABLE 1B). Genes in the inflammation category exhibited some of the most robust monotonic changes with aging seen in our results using the method of the invention (*e.g.*, most were significant at the $p<0.001$ criterion with 0.025 FDR) (TABLE 1B). Moreover, most were inversely correlated with cognitive function (FIG. 3B).

[55] Consistent with evidence of a role for oxidative stress in brain aging (Carney JM *et al., Proc Natl Acad Sci U S A 88:* 3633-6 (1991), Hensley K *et al., Ann N Y Acad Sci 786:* 120-34 (1996), Bickford PC *et al., Brain Res 866:* 211-7 (2000), Lee CK, *et al. Nat Genet 25:* 294-7 (2000), Jiang CH *et al., Proc Natl Acad Sci U S A 98:* 1930-4 (2001)), we also found increased expression for molecules important in defense against oxidative stress (GST, GSTa1) (TABLE 1B). One potentially key new finding here, as noted above, was that DHPR was upregulated with aging and correlated with cognitive decline (TABLE 1B). Its product, tetrahydrobiopterin, is also an essential cofactor for nitric oxide synthase (Boyhan A, *et al. Biochem J 323 (Pt 1)* 131-9 (1997)). Because oxyradicals formed from nitric oxide appear to play a major role in inflammatory neuronal damage (Bal-Price A & Brown GC, *J Neurosci 21:* 6480-91 (2001), Calingasan NY & Gibson GE, *Brain Res 885:* 62-9 (2000)), this may be an important pathway through which the deleterious effects of inflammation are mediated in brain aging.

[56] *Glial Markers.* Astrocyte reactivity and astrocyte markers are also well recognized to increase in the aged rodent and human hippocampus (Landfield PW *et al., Science 214:* 581-4 (1981), Landfield PW *et al., J Neurobiol 23:* 1247-60 (1992), Nichols NR *et al., Neurobiol Aging 14:* 421-9 (1993), Finch CE & Longo VD, *Neuroinflammatory Mechanisms in Alzheimer's Disease: Basic and Clinical Research,* 237-256 (2001)) and the present data confirm extensive upregulation of genes (Finch CE & Tanzi RE *Science 278:* 407-11 (1997)) for glial markers (*e.g.*, vimentin, GFAP - cytoskeleton category, TABLE 1B). In addition, we extended those observations to show that genes for proteoglycans (TABLE 1B) and other extracellular proteins (*e.g.*, fibronectin) that are components of astroglial scars also were upregulated. These changes may reflect astroglial-mediated reorganization of the extracellular matrix, a process known to be unfavorable for axonal remodeling.

[57] *Signal Transduction.* Several genes in calcium regulating and G-protein-coupled signaling pathways were also identified (TABLE 1B). In particular, S100A1, which

modulated Ca^{2+}-induced Ca^{2+} release, and PI 4-kinase, which acts to produce IP3 were upregulated. Several other S100-related genes (*e.g.*, S100A4 and P9K2; TABLE 3) were also upregulated with aging but failed to meet the strict criteria set forth herein (FIG. 2).

[58] *Biosynthesis*. Concomitantly, many ribosomal (growth) and protein processing genes were upregulated (TABLE 1B). The upregulated changes reflect increased protein synthesis, turnover and phagocytosis associated with strongly elevated biosynthetic processes in glial compartments (*e.g.*, elevated myelin, MHC, proteoglycan synthesis).

[59] *Orchestrating Factors*. Our data show that a number of transcriptional regulators and cytokines, including KZF-1, Roaz and members of the NFI family (TABLE 1B) were upregulated and therefore, may be strong candidates for coordinating factors. Under some conditions, several of these factors function as negative transcriptional regulators.

[60] *Relationship to Fold Change*. The large majority of microarray analyses to date have used fold-change criteria to detect changes in expression. In addition to providing little basis for statistical assessment (*e.g.*, Miller RA, *et al. J Gerontol A Biol Sci Med Sci 56* B52-7 (2001)), however, fold-change criteria are relative insensitive. Among the 139 ACGs, most exhibited group mean fold changes between the Young and Aged groups of less than 1.5 (92), a few showed fold changes between 1.5 and 2.0 (26), and only a handful of genes exceeded 2-fold-change (20) (TABLES 1A and B). Thus, few of our results using the method of the invention would have been detected in the great majority of prior microarray studies, in which 1.7 to 2-fold change cutoffs are commonly used as minimum criteria for identifying differences, and many changes are reported in the 3 - 4 fold range. Further, the rank order correlation between group mean fold-change and p values on the ANOVA for all aging-significant genes, although significant, was modest according to Spearman's correlation test (Spearman's $r = 0.45$, $p < 0.001$). Armitage P & Berry G, *Statistical Methods in Medical Research, 2nd Edn.*, 200-205 (1987). This indicates that fold-change accounted for only ~ 20% of the variance (r2) in the degree of statistical significance on the ANOVA. Some of our results detected with the enhanced sensitivity of statistical analysis were extremely subtle (*e.g.*, 1.1 fold for the L28 and L29 ribosomal proteins, TABLE 1B). Despite this enhanced sensitivity, however, numerous false negatives were still undoubtedly present in our data set.

[61] *Age Course of Gene Expression Changes*. Using a design with three age groups enabled us to classify genes and categories according to their general patterns of age dependence of change (FIGS. 4 and 5). Genes were classified by whether 75% of the

maximal change occurred between the Young and Mid-Aged groups (Yng to Mid), the Mid-Aged and Aged groups (Mid to Aged), or the Young and Aged groups (monotonic).

[62] Almost all categories comprising downregulated and cognitively correlated genes (TABLE 1A), exhibited their greatest change between the Young and Mid-Aged points, and many did not show much additional downregulation between the Mid-Aged and Aged groups (FIG. 4). This was also true for the entire population of genes whose expression decreased with aging at $p < 0.025$ (pie-chart inset, FIG. 4). Conversely, by far the largest fraction of functional categories of upregulated genes showed a monotonic age course of change that also began between the Young and Mid-Aged points but, in addition, continued between the Mid-Aged and Aged points (FIG. 5). However, the Cytoskeletal and Transcriptional Regulator categories contained significant numbers of exceptions that exhibited >75% of their change between the Young and Mid-Aged groups (TABLE 1B). Additionally, among all genes that showed significant upregulation with aging, the majority fit the monotonic classification (pie-chart inset, FIG. 5). Only a few scattered genes showed a predominantly Mid to Aged change pattern (*e.g.*, FIGS. 4 and 5 pie-charts).

[63] *Strongest Correlations of Pathways with Memory Performance.* To determine which pathways were most closely correlated with memory performance, we calculated the percentage of genes in each of our categories that were correlated significantly (at $p < 0.025$) with both memory tests. We reasoned that each test measures aspects of memory but each test also has its own error sources and confounding contributions from non-cognitive performance factors. Therefore, genes that correlated with both tasks seem more likely to be associated with cognitive processes.

[64] Because memory performance changed most between the Mid-Aged and Aged groups (FIG. 1), whereas downregulated genes changed little (FIG. 4) and upregulated genes continued to increase (FIG. 5) between those groups, the pattern of age course changes relative to cognitive performance was more similar for upregulated than for downregulated genes. Not surprisingly, therefore, more upregulated (52%) than downregulated (44%) genes were correlated with performance on both tasks. Three categories of downregulated genes had 50% or higher both-task correlations: Adhesion and extracellular matrix (3/5), Metabolism (8/10), and Protein processing and trafficking (3/5). Whereas seven categories of upregulated genes had 50% or higher both-task correlations: Signaling (5/7), Inflammation (14/20), Cytoskeleton/Vesicle (3/6), Myelin related proteins (3/5), Amino acid/ transmitter

metabolism (2/4), Transporters and carriers (3/5), and Growth, biosynthesis, maintenance (7/12). In the Signaling category, moreover, genes involved in intracellular Ca^{2+} release, S100A1 and PI3-K (TABLE 1B), were correlated with both tasks.

[65] Another way to examine closeness of correlation specifically with memory impairment is to correlate gene expression with performance only in the aged group. This correlation focuses on variation in the performance of aged animals and removes the overall age course pattern from contributing to the correlation with impairment. This correlation is independent of the ANOVA for aging effects and an FDR also can be calculated. Consequently, we tested each of the 139 primary aging- and behaviorally-related genes for correlation with 24 hr memory performance on the OMT in the aged group. The OMT was selected over the SWM for this test as it had the greater dispersion of performance needed for correlation analysis. The correlation tests in the aged group ($n = 10$) of course had considerably less power than across all three groups ($n = 29$) and the criterion for significance was set at $p < 0.025$.

[66] Only 3 (4.9%) of the downregulated ACGs, but 10 (12.2%) of the upregulated ACGs were correlated with Aged group performance on the OMT. The FDR for these genes was 0.28. Two of the 3 downregulated ACGs were accounted for by the Synaptic Structural Plasticity category (Fez-1, agrin). For upregulated genes, two of the 10 ACGs were from Inflammation (MHC and CD59 antigen), three from Cytoskeleton/Vesicle category (Vcp, rsec8 and p41-Arc), and three from Growth/Biosynthesis (2 ribosomal proteins and CD24 antigen). No other category had more than one, including Transcription (NF1-A) and Protein processing and trafficking (Skd3).

[67] Thus, by the criterion of correlation on both tasks, the upregulated categories of Inflammation/Immune, signaling (particularly Ca^{2+} signaling), Cytoskeleton/Vesicle and Amino Acid Metabolism were ranked most highly. By the criterion of correlation in the aged group only, the upregulated categories of Cytoskeleton/Vesicle (3/6), Biosynthesis (3/12) and Inflammation (2/20), and the downregulated category of Synaptic Plasticity (2/7) were ranked most highly.

[68] *Benefits of the Invention.* One of the major problems associated with developing treatments for aging-dependent functional decline is the lack of good genomic biomarkers or targets of brain aging needed for evaluating the efficacy of different treatments. Our ACGs, therefore, could serve as excellent biomarkers of cognitive aging. Using microarrays

constructed to contain oligonucleotide sequences specific for hybridization with and measurement of mRNAs of the identified ACGs, laboratory animals could be assessed for degree of cognitive aging before, during and after treatment with a compound. Treatments that slowed or reversed the ACG profile during aging might be highly promising for development as new therapeutic approaches. Further, treatments that slowed or reversed expression profiles of particular genes in our panel of biomarkers might reveal which specific genes among the subset of ACGs are most critical for the age-dependent functional decline and, therefore, would suggest genes and gene products that should be targeted with high priority for development of therapeutic interventions. The same approach could be applied using our panel of unique brain aging genes that are not specifically clustered with cognition related genes, to evaluate and develop new therapies and compounds for treatment of brain aging in general.

[69] The panel of ACGs identified here can be used on a microarray to perform diagnostic tests. Subjects suspected of having accelerated brain aging or early age-related neurodegenerative disease could provide a small brain biopsy sample for testing by microarray. This could then determine the subject's suitability for pharmacologic intervention.

[70] Based on the gene lists described above, investigators can develop new drugs or treatments aimed at altering the activity of one or more genes in the lists, or products encoded by those genes, or targets of the products, with the goal of counteracting age-related cognitive impairment or brain aging in general.

[71] A smaller subset of ACGs, specifically linked to some process or system (*e.g.*, to inflammation, mitochondrial function, or lipid metabolism, *etc.*), could be used in a microarray to test efficacy of a new compound targeted to slowing or reversing aging and cognitive changes dependent on that set of genes or gene-impacted systems, either in experimental tests to develop new compounds, or as diagnostic or therapeutic guides.

[72] *Relevance to Human Brain Aging and Alzheimer's Disease.* Normal human brain aging is associated with memory dysfunction and appears to set the stage for Alzheimer's disease and other age-related neurodegenerative conditions. It also shares many features with animal models of aging. Landfield PW *et al.*, *J Neurobiol 23*: 1247-1260 (1992). Thus, many of the memory-correlated gene expression profiles seen here in rats may have implications for genomic mechanisms of human brain aging and/or Alzheimer's disease. This view is

supported by several parallels between processes identified here and those seen in human aging or Alzheimer's disease. For example, myelin abnormalities are also found extensively in normal brain aging in humans (leukoaraiosis). These white matter changes in humans are also correlated with cognitive dysfunction and become more severe in disease states. Further, cerebral metabolism begins to decline by mid-life in humans, much as it apparently does in rats (FIG. 4). Of particular note in light of our findings on oxidative phosphorylation and myelin turnover, mitochondrial diseases in humans also can result directly in demyelination.

[73] It is interesting, in view of the apparently altered lipid metabolism seen here, that activity of the cholesterol ester synthesizing enzyme acyl CoA:cholesterol acyltransferase (ACAT) is elevated in Alzheimer's disease and appears directly coupled to amyloid production. ACAT has lipogenic functions somewhat similar to those of LCAT, which was also upregulated here (TABLE 1A). Moreover, activity of glycerol-3-phosphate dehydrogenase (GPDH) is elevated in association with abnormal glucose metabolism in brains of patients with Down's syndrome. The gene encoding this glycolytic enzyme was also upregulated here (TABLE 1A). Other processes found in human aging or Alzheimer's disease brain for which we found corollaries in gene expression include, as noted, inflammation, oxidative stress and elevated KatII (kynurenine aminotransferase 2), among others. Thus, if these parallels depend, at least in part, on similar mechanisms, our our results show that widespread genomic regulatory changes would reasonably be expected to contribute to altered cerebral metabolism, lipid synthesis, neural activity and myelination in human brain aging as well.

[74] *Implications for a New Hypothesis of Brain Aging.* Based on the functional implications of our results, as discussed above, we provide a new working model of brain aging (FIG. 6). Early in adult life (*i.e.*, before mid-life) a series of brain changes begin, perhaps initiated by new expression of genes that exert deleterious late-life actions (*e.g.*, "late genes") (Finch CE, *Longevity, Senescence and the Genome,* 37-42 (Univ. Chicago Press, Chicago, 1990); Austad, SN, *Why We Age: What Science Is Discovering about the Body's Journey Through Life* (Indianapolis, Wiley, 1999)) or by catabolic hormonal processes (*e.g.*, glucocorticoids, Porter NM & Landfield PW, *Nature Neurosci 1*: 3-4 (1998)). These changes include reduced neuronal activity and induce a subtle shift from anabolic to catabolic metabolism in neurons. In neurons, the reduced anabolic capacity leads to diminished capacity for protein biosynthesis and, in particular, for activity-dependent neurite remodeling

and synaptogenesis. Concomitantly, an increase in degradation of myelin and lipids begins, perhaps triggered by reduced neural activity, or reduced oxidative phosphorylation and/or demand for an alternative energy source, or by an immune process similar to multiple sclerosis, among other possibilities. The degenerating myelin fragments are endocytosed in microglia and astrocytes, degraded by lysosomes and packaged into antigen-presenting MHC molecules. This in turn activates orchestrating cytokines and transcription factors that trigger an inflammatory reaction in the glia and possibly, in macrophages. The inflammation further accelerates the phagocytosis and degradation of myelin. As astrocytes hypertrophy, they increase glycolytic metabolism and synthesize "glial scar" proteins (*e.g.*, fibronectins, proteoglycans) that alter the extracellular matrix. In oligodendrocytes, lipogenic and myelin synthesis programs are activated in response to the ongoing demyelination and/or altered signaling pathways. In turn, remyelination may increase demand for lipid substrate and thereby also accelerate demyelination. Thus, positive feedback cycles between demyelination and myelination and/or between demyelination and inflammation, among other processes, might develop and further drive cellular dyshomeostasis. Eventually, the reduced synaptogenic capacity unfavorable extracellular matrix and degradative inflammatory processes result in failure of cognitive processing. Additionally, the ongoing catabolic processes erode neuronal membranes and cytoskeletons, increase protein aggregation and enhance vulnerability to neurodegenerative disease. Accordingly, our results, in conjunction with this working model, point directly to potentially useful therapeutic interventions and should, therefore, facilitate the design of such future therapeutics.

[75] The details of one or more embodiments of the invention are set forth in the accompanying description above. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. Other features, objects, and advantages of the invention will be apparent from the description and from the claims. In the specification and the appended claims, the singular forms include plural referents unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All patents and publications cited in this specification are incorporated by reference.

[76] The following EXAMPLES are presented in order to more fully illustrate the preferred embodiments of the invention. These examples should in no way be construed as limiting the scope of the invention, as defined by the appended claims.

EXAMPLE 1

BEHAVIORAL RESULTS

[77] Thirty animals in three age groups (n = 10/group) were trained sequentially on two tasks, first in the Morris spatial water maze (SWM) and then in the object memory task (OMT). Male Fischer 344 rats aged 4 months (Young, n = 10), 13 months (Mid-Aged, n = 10) and 24 months (Aged, n = 10) were used. Overall, the training/testing lasted seven days, and hippocampal tissue was collected 24 hr later. Training or testing occurred on each day except for the 2nd and 3rd days of the seven-day sequence.

[78] Methods used here for cognition assessment in the Morris Spatial Water Maze (SWM), a task sensitive to both hippocampal function and aging, have been described previously Norris CM & Foster TC, Neurobiol Learn Mem 71, 194-206 (1999). Briefly, rats were trained in a black tank, 1.7 M in diameter, filled with water (27±2° C). Behavioral data were acquired with a Columbus Instruments tracking system. After habituation to the pool, animals were given cue training with a visible platform (five blocks of three trials, maximum of 60 sec/trial, 20 sec intertrial interval and a 15 min interval between blocks). Rats remained in home cages under warm air after each block. Cue training was massed into a single day and the criterion for learning was finding the platform on 4 of the last 6 trials. For all animals that met this criterion, spatial discrimination training was initiated three days later in which the escape platform was hidden beneath the water but remained in the same location relative to the distal cues in the room. Fifteen min following the end of spatial training, a 1-min duration free-swim probe trial with the platform absent was administered, during which crossings over the former platform site (platform crossings) were recorded to test acquisition, followed by a refresher training block. Retention for platform location was again tested 24-hr later using a second 1-min free-swim probe trial.

[79] During cue training in the SWM, all animals were able to locate the visible escape platform according to our criteria and therefore, were trained on the hidden platform spatial task. During acquisition, Aged animals performed more poorly (longer latencies) than Mid-Aged or Young. In addition, an aging-dependent decrease in 24 hr retention, measured

by platform crossings (1-way ANOVA, $p < 0.05$), was observed on the retention probe trial (FIG. 1A). *Post hoc* analysis indicated that Young and Mid-Aged animals exhibited more platform crossings relative to Aged animals, but did not differ from each other.

[80] Methods used here for cognition assessment in the object memory task OMT) have been described previously by Ennaceur A & Delacour J, *Behav Brain Res 31*: 47-59. (1988). The object memory task (OMT) is also both sensitive to hippocampal function and affected by aging but is less dependent on physical strength and endurance. On the afternoon of the final spatial maze probe trial, animals were administered a habituation session (15-min) in the empty mesh cage to be used for the OMT (63.5 cm X 63.5 cm). OMT training began 24 hr after habituation and consisted of a 15-min acquisition session during which two 3-dimensional objects were placed at opposite sides of the cage, followed by two 15-min retention test sessions at 1 and 24 hr posttraining. During the acquisition session, the cage contained two sample objects (A and B) and the time spent actively exploring each object was recorded. After 1 hr, the rat was reintroduced into the cage and the time spent exploring a novel object, C, relative to the familiar object, B, was recorded. On the 24 hr test, familiar object A was reintroduced and object B was replaced by a second novel object, D. Objects were randomized across individuals and timed measures of exploration were used to calculate a memory index (MI) as follows: MI = (N-F)/T, where *N* is time spent exploring the novel object, *F* is time spent exploring the familiar object, and *T* is total time spent exploring the two objects. More time spent exploring the novel object (higher MI) is considered to reflect greater memory retention for the familiar object.

[81] In the OMT, Aged animals performed as well as Young or Mid-Aged on the 1 hr retention test (not shown), but there was a significant age-related decline in recall (1-way ANOVA, $p<.001$, for the main effect of age) on the 24 hr test (FIG. 1B). At 24 hr, Young and Mid-Aged groups were significantly different from the Aged group, but not from one another (Young vs. Aged: $p<.001$; Mid-Aged vs. Aged: $p<.05$; Young vs. Mid-Aged: N.S., Tukey's *post-hoc* test; Armitage P & Berry G, *Statistical Methods in Medical Research, 2nd Edn.*, 200-205 (1987)).

EXAMPLE 2

GENE MICROARRAY CHIP RESULTS

[82] Microarray analyses were performed on hippocampal CA1 tissues from each of the same behaviorally characterized 30 animals (one chip per animal), but one chip was lost for technical reasons, leaving a data set of 29 microarrays (Young = 9, Mid-Aged = 10, Aged = 10). For tissue preparation, twenty-four hours after completion of the OMT testing, animals were anesthetized with CO_2 gas and decapitated. The brains were rapidly removed and immersed in ice-cold, oxygenated artificial cerebrospinal fluid consisting of (in mM): 124 NaCl, 2 KCl, 1.25 KH_2PO_4, 2 $MgSO_4$, 0.5 $CaCl_2$, 26 $NaHCO_3$, and 10 dextrose. Hippocampi were removed and the CA1 region from one hippocampus per animal were dissected by hand under a stereomicroscope. The CA1 tissue block from each animal was placed in a microcentrifuge tube and flash frozen in dry ice for RNA isolation.

[83] For RNA isolation, total RNA was isolated using the TRIzol reagent and following the manufacturer's RNA isolation protocol (Gibco BRL, #15596). One ml of TRIzol solution was added to each tube containing the frozen tissue block and the tissue was homogenized by 10 passages through an 18 ½ G syringe needle. After centrifugation, the RNA was precipitated from the aqueous layer, washed and redissolved in RNase-free water. RNA concentration and the integrity of RNA were assessed by spectophotometry and gel electrophoresis. The RNA samples were stored at –80°C.

[84] Gene expression analyses were performed using the Affymetrix GeneChip System. The labeling of RNA samples, rat GeneChip (RG-U34A) hybridization and array scanning were carried out according to the Affymetrix GeneChip Expression Analysis Manual (r.4.0, 2000). Each animal's CA1 RNA was processed and run on a separate rat gene chip. Briefly, an average yield of 40 μg biotin-labeled cRNA target was obtained from 5 μg of total RNA from each CA1 sample, of which 20 ug cRNA was applied to one chip. The hybridization was run overnight in a rotating oven (Affymetrix) at 45^{0}C. The chips were then washed and stained on a fluidics station (Affymetrix) and scanned at a resolution of 3 μm in a confocal scanner (Agilent Affymetrix GeneArray Scanner).

[85] Each U34A rat chip (Affymetrix, Santa Clara, CA) contained 8,799 transcript probe sets (gene representations). Although the measured signal intensity for a transcript probe set (Methods) reflects mRNA content, it is referred to here as "gene expression". However, it is

well recognized that mRNA stability and other factors in addition to gene transcription can affect mRNA content.

[86] We used the microarray suite (MAS 4.0) software (Affymetrix) to calculate the overall noise of the image (the amount of variation around the mean intensity, Qraw) for each array. Overall noise was highly similar across arrays in all 3 age groups (Young: 21.81±1.55; Mid Aged: 21.25±2.24; Aged: 20.66±2.06, N.S.). "All probe set scaling" was used to set overall intensities of different arrays to an arbitrary target central intensity of 1500. Thus, the average intensity of each array was adjusted to the 1500 value using a scaling factor (SF). There was no significant difference in SF across ages (Young: 1.58±0.14; Mid Aged: 1.46±0.20; Aged: 1.63±0.16, N.S.).

[87] The algorithm used to determine Presence/Absence is listed in the Microarray Suite 4.0 Manual and is the basis upon which a particular transcript is determined to be reliably detectable by a given probe set. Average difference scores, the average of the difference in expression intensity (ADEI) of each probe pair within a probe set, formed the basis for determining expression (relative abundance) of transcripts, and throughout the text the term "expression level" refers to the ADEI score. When comparing across appropriately normalized arrays, the larger the ADEI score, the greater the relative expression for that particular message. However, ADEI scores are not comparable for relative expression levels among different messages on the same chip, as there are several other factors that can confound such an assessment (*e.g.*, p. 356, Affymetrix Microarray Suite 4.0 User's Guide).

[88] The Presence/Absence calls and Average Difference scores for all probe sets on all 29 arrays were then copied from the MAS pivot table to an Excel 9.0 (Microsoft, SR-1) workbook. From within Excel, the following data manipulations were performed.

[89] *Min-Max:* For the purposes of filtering (FIG. 2), each probe set was normalized according to the formula: $x' = \frac{x - \overline{X}\min}{X\max - X\min}$, where *x is ADEI score,* $\overline{X}_{min}$ is the mean for the age group with the lowest ADEI score, and $\overline{X}_{max}$ is the mean for the age group with the largest ADEI score. Thus, normalized mean values varied between 0 (lowest) and 1 (highest) for each probe set.

[90] *Standardization (Z-score)*: For the purpose of obtaining the mathematical means within functional categories and graphing, the data was normalized using the Z-score method:

$z = \frac{x - \overline{X}}{SD(x)}$, where $\overline{X}$ is the mean, and *SD(x)* is the standard deviation of ADEI across all age groups for an individual probe set.

[91] *Statistical Analysis.* All statistical tests were performed using a combination Excel (Microsoft, version 9, SR-1) and Sigma Stat (SPSS, version 2).

EXAMPLE 3

MULTI-STEP GENE IDENTIFICATION ALGORITHM

[92] The analytic algorithm of the invention, which addresses the bioinformatics issues noted above, comprise three main steps aimed first, at reducing the number of comparisons (to manage type I error), second, at reliably detecting modest aging differences with global statistical analyses (by ANOVA), and third, at identifying aging-related expression changes that were quantitatively correlated with cognitive function (by Pearson's test; Armitage P & Berry G *Statistical Methods in Medical Research, 2nd Edn.*, 200-205 (1987)) (*see*, FIG. 2).

[93] *Multiple Comparison Reduction Step.* The expected false positives in a series of multiple comparisons (false positive rate) are predicted to be a percentage of the total statistical comparisons to be made, as defined by the *p-value* (*i.e.*, tests at $p < 0.05$ will on average generate 5% false positives). Accordingly, the absolute numbers of expected false positives can be decreased simply by reducing the total transcript sets that are tested in a microarray analysis. This can be done by deleting all transcripts identified *a priori* as not likely to be relevant to the specific interests of the analysis.

[94] Using this step of the method, we reduced the total transcripts to be tested in three phases. In the first phase, we deleted quality control oligonucleotide sets ("control", $n = 60$) and all gene transcripts (probe sets) rated "absent" by our criteria. As used in this specification, the term "quality control oligonucleotides" are those oligonucleotides and polypeptides used to test for the appropriate behavior of the technological system, rather than to measure expression levels of biological interest.

[95] Of the original 8,799 sets, 4,118 gene transcript sets were removed at this stage, leaving 4,681 transcript sets that were called "present" for further consideration (FIG. 2, step 1a). In the second phase, we deleted all "present" transcript sets representing "expressed sequence tags" (ESTs), which have not yet been clearly linked to known genes (FIG. 2, step 1b). There were 1,213 such ESTs rated "present" that we filtered out in this phase, leaving

3,468 transcript sets for further consideration. The third reduction phase was based on our interest in persistent aging-dependent changes reflected in substantial differences between the youngest and oldest groups. We further decreased the total transcript sets to be tested by deleting sets in which the difference between the Young and the Aged group did not comprise at least 75% of the maximum normalized difference among groups (*i.e.*, in which age-related changes from the Young baseline values were maximal in the Mid-Aged group, but then reversed substantially (>25%) in the Aged group, possibly because of random, compensatory or developmental factors). There were 1,483 sets removed by this criterion, retaining 1,985 probe sets of the original 8,799 for formal statistical testing (FIG. 2; step 2). If the original 8,799 sets had been tested at the $p < .025$ alpha level, ~420 false positives would have been expected. However, by reducing the total number of sets to be tested for statistical significance (at $p < 0.025$), we reduced the absolute numbers of false positives expected from multiple tests, to ~50 (5% of 1985).

[96] *Group Statistical Testing Step (ANOVA).* In this second main step of the algorithm, each of the remaining1,985 transcript sets was tested by 1-way ANOVA for a significant effect of aging (at $p \leq 0.025$) across the 3 age groups (n = 9-10/group). Of the 1,985 tested sets, 233 were found to change significantly with aging (observed total positives). As noted, at $p < 0.025$, approximately 2.5% (~50) of the 1,985 tested should be significant by chance alone (expected false positives). In order to estimate the proportion of false discoveries anticipated among our 233 observed positives (*i.e.*, the fraction of observed positives expected to be false), we used the expected false positive value to calculate the false discovery rate (FDR) (Benjamini *et al., Behav Brain Res 125*: 279-284 (2001)). For any multiple comparison, the false discovery rate provides an empirical estimate of the anticipated chance error rate among all positives detected. It is partly analogous to the *p value* of statistical tests, in that the false discovery rate yields the probability that any positive found at the alpha level used (in this case $p < 0.025$) is positive by chance alone.

[97] For the ANOVA-positive results, the FDR was 50/233 = 0.21, indicating that up to 21% of the observed positives might be positive by chance alone or, that any one positive had a 21% chance of being a false positive.

[98] In addition, we examined the FDR obtained using two other ANOVA *p-value* levels, $p < 0.01$ and $p < 0.001$. At the $p < .01$, ~ 20 genes should be found positive by chance alone among the 1,983 transcripts tested. A total of 145 total positives were observed, yielding an

FDR of 20/145 = 0.14. At $p < 0.001$ only 2 false positives are expected in 1,983 tests, and 70 total positives were found. This yields a FDR of 2/70 = .03. The latter, in particular, compares highly favorably with the 0.05 alpha level conventionally accepted for statistical significance in univariate analyses.

[99] However, as noted, additional confidence and validation is gained in microarray analyses when similar patterns of regulation are found among multiple functionally similar genes (Prolla *et al., J Gerontol A Biol Sci Med Sci 56*: B327-330 (2001)). This is because such genes are not necessarily independent and their co-regulation can provide added cross-validation (*e.g.*, Mirnics *et al., Neuron 28*: 53-67 (2000); Prolla *et al., J Gerontol A Biol Sci Med Sci 56*: B327-330 (2001)). Consequently, in many cases, confidence advantages can be gained by relaxing *p-value* criteria in order to expand the numbers of genes included in functional categories. Mirnics K, *Nat Rev Neurosci 2:* 444-447 (2001). Further, relaxing stringency of the *p-value* reduces the likelihood of type II error (false negatives). Based on these rationales, we used the set of 233 genes obtained at the less stringent $p \leq 0.025$ alpha level (rather than the set of 70 at $p \leq 0.001$) for the next main step of our algorithm, the behavioral correlation analysis (FIG. 2, step 3a).

[100] *Cognitive Performance Correlation Step (Pearson's Test).* In this step we identify a specific subset of the 233 aging-significant (by ANOVA) genes that was also correlated with memory performance in both the OMT and SWM. We tested each of the 233 ANOVA-significant genes across animals for statistical correlation between that gene's expression value and behavioral scores (with Pearson's test).

[101] The expression of 161 of the 233 ANOVA-significant genes was correlated significantly with behavioral performance on memory-dependent tasks ($p \leq 0.025$; ACGs). Of these, 84 were significantly correlated with both OMT and SWM performance, 40 were significantly correlated with OMT, and 37 were significantly correlated with SMW (FIG. 2, step 3a). Of the 233 genes significant by ANOVA across age, 72 were significantly correlated with neither OMT nor SWM. Of these, 11 were significant by ANOVA across age at the more stringent p-value of $\leq .001$ (FIG. 2, step 3b) and were included for further analysis. Of the 161 ACGs, 64 exhibited decreased expression with aging and 97 exhibited increased expression with aging. Examples of the correlation patterns with behavior in the Aged group for the genes with the five highest correlations in each direction are shown in FIG. 3.

[102] Because of the voluminous literature involved, many relevant citations are not included here. In addition to the "dual function" status of some genes, the functions of many are not completely understood, and therefore, the categorization here, while generally consistent with published reports, is not definitive.

[103] ACGs that were downregulated with aging (TABLE 1A) appeared primarily to represent metabolic and neuronal functions. A substantial number of them fell into the category of oxidative metabolism (TABLE 1A). Many also fell into categories of synaptic/neuritic remodeling or other activity-dependent neuronal processes, *e.g.*, immediate early genes (IEGs) (TABLE 1A). Conversely, ACGs that were upregulated with aging fit primarily into categories that appeared to reflect activated inflammatory response (TABLE 1B).

[104] Additionally, among the main unexpected findings was a widespread upregulation in the expression of multiple genes encoding proteins for myelin synthesis (TABLE 1B) and lipid turnover (TABLE 1B). These various categories, overall, are consistent with a downward shift of oxidative metabolism in parallel with a major upregulation of lipid metabolism.

EXAMPLE 4

GENES IDENTIFIED BY THE METHOD OF THE INVENTION

[105] The following tables provide additional results from the tests performed above, and supplement the results presented in TABLES 1A and B.

TABLE 2

ESTs That Were Aging And Cognition Related or Showed Highly Significant Age-Dependent Changes in Expression Level

GenBank	Description	Young	Mid	Age	FC	ANOVA p
Decreased with Age						
	Correlated with both OMT and SWM					
AA963449	UI-R-E1-gj-e-08-0-UI.s1 cDNA	2499 ± 80	2122 ± 102	1874 ± 37	-1.33	0.0000
AA892532	EST196335 cDNA	4156 ± 85	4194 ± 80	3715 ± 100	-1.12	0.0010
AA859626	UI-R-E0-bs-h-02-0-UI.s1 cDNA	853 ± 22	705 ± 23	714 ± 35	-1.20	0.0013
AA893743	EST197546 cDNA	2292 ± 63	1985 ± 80	1846 ± 92	-1.24	0.0022
AI233365	EST230053 cDNA	8460 ± 232	7572 ± 289	7151 ± 226	-1.18	0.0042
H31665	EST105952 cDNA	1160 ± 56	1017 ± 34	942 ± 38	-1.23	0.0051
AA892353	ESTs, Moderately similar to JC5823 NADH dehydrogenase	890 ± 59	796 ± 66	602 ± 47	-1.48	0.0054
AI639247	mixed-tissue library cDNA clone rx03939 3	945 ± 36	814 ± 45	749 ± 36	-1.26	0.0063
AA858617	UI-R-E0-bq-b-06-0-UI.s1 cDNA	397 ± 17	294 ± 32	285 ± 22	-1.39	0.0072
AI639429	mixed-tissue library cDNA clone rx00973 3	341 ± 31	350 ± 22	252 ± 21	-1.35	0.0148
AA858620	UI-R-E0-bq-b-09-0-UI.s1 cDNA	153 ± 24	93 ± 10	86 ± 14	-1.78	0.0160
	Correlated with OMT					
AA866291	UI-R-A0-ac-e-12-0-UI.s3 cDNA	13818 ± 281	12477 ± 171	11987 ± 406	-1.15	0.0008
AA894104	EST197907 cDNA	5716 ± 164	5259 ± 156	4871 ± 179	-1.17	0.0060
AA799996	EST189493 cDNA	4881 ± 67	4812 ± 110	4407 ± 120	-1.11	0.0066
AA892805	EST196608 cDNA	6563 ± 147	6174 ± 247	5645 ± 212	-1.16	0.0176
AI639019	mixed-tissue library cDNA clone rx01107 3	353 ± 19	315 ± 24	265 ± 16	-1.33	0.0188
AA799538	EST189035 cDNA	1436 ± 156	1337 ± 76	963 ± 117	-1.49	0.0211

TABLE 2

ESTs That Were Aging And Cognition Related or Showed Highly Significant Age-Dependent Changes in Expression Level

GenBank	Description	Young	Mid	Age	FC	ANOVA p
Decreased with Age						
	Correlated with SWM					
AI070108	UI-R-Y0-lu-a-09-0-UI.s1 cDNA	1542 ± 36	1327 ± 39	1307 ± 58	-1.18	0.0022
AA866409	UI-R-E0-ch-a-03-0-UI.s1 cDNA	994 ± 38	814 ± 37	819 ± 35	-1.21	0.0026
AA859632	UI-R-E0-bs-h-08-0-UI.s1 cDNA	415 ± 53	352 ± 17	247 ± 18	-1.68	0.0040
AA891651	EST195454 cDNA	16635 ± 723	15405 ± 589	13530 ± 521	-1.23	0.0051
AA893032	ESTs, Moderately similar to CALX calnexin precursor	606 ± 26	491 ± 30	501 ± 17	-1.21	0.0060
AA891965	EST195768 cDNA	2353 ± 55	2260 ± 60	2088 ± 45	-1.13	0.0060
AA800708	ESTs, Weakly similar to S28312 hypothetical protein F02A9.4	1042 ± 38	945 ± 43	805 ± 58	-1.29	0.0065
AA964320	UI-R-C0-gu-e-09-0-UI.s1 cDNA	18110 ± 355	17683 ± 319	16605 ± 293	-1.09	0.0082
AA893173	EST196976 cDNA	9712 ± 294	8674 ± 503	8155 ± 222	-1.19	0.0196
H32977	EST108553 cDNA	3159 ± 74	2640 ± 85	2698 ± 66	-1.17	0.0001
AA874887	UI-R-E0-ci-g-10-0-UI.s1 cDNA	459 ± 43	284 ± 23	316 ± 11	-1.45	0.0004
AA850781	EST193549 cDNA	1886 ± 54	1570 ± 55	1602 ± 49	-1.18	0.0004
Increased with Age						
	Correlated with both OMT and SWM					
AI176456	ESTs, Weakly similar to endothelial actin-binding protein	8156 ± 447	9404 ± 462	12460 ± 511	1.53	0.0000
H31418	EST105434 cDNA	1176 ± 92	1530 ± 66	1904 ± 83	1.62	0.0000
AA858588	ESTs, Weakly similar to dihydrolipoamide acetyl transferase	2740 ± 80	2824 ± 86	3466 ± 198	1.26	0.0014
AA891785	EST195588 cDNA	1140 ± 122	1299 ± 82	1675 ± 89	1.47	0.0021
AA799803	ESTs, Weakly similar to K1CU cytoskeletal keratin (type 1)	149 ± 35	227 ± 28	297 ± 20	1.99	0.0035
AA799449	EST, Weakly similar to ubiquitin carboxyl-terminal hydrolase 4	-80 ± 7	-2 ± 26	17 ± 19	1.00	0.0044
	Correlated with OMT					
AA859777	UI-R-E0-bu-e-10-0-UI.s1 cDNA	1001 ± 43	1396 ± 76	1437 ± 87	1.44	0.0004
AI639532	mixed-tissue library cDNA clone rx01030 3	209 ± 16	282 ± 18	317 ± 22	1.52	0.0018
AA875059	UI-R-E0-cb-f-04-0-UI.s1 cDNA	233 ± 20	219 ± 12	297 ± 14	1.28	0.0023
AI012051	EST206502 cDNA	786 ± 68	987 ± 58	1200 ± 101	1.53	0.0042
AA800549	EST190046 cDNA	3647 ± 121	4078 ± 223	4573 ± 231	1.25	0.0132
	Correlated with SWM					
AA799854	EST189351 cDNA	211 ± 49	328 ± 46	487 ± 60	2.31	0.0037
AA892520	EST196323 cDNA	834 ± 38	826 ± 29	960 ± 36	1.15	0.0152
AA893607	EST197410 cDNA	-9 ± 19	69 ± 20	122 ± 22	1.99	0.0006
AI639381	mixed-tissue library cDNA clone rx01495 3	1531 ± 148	2417 ± 152	2353 ± 189	1.54	0.0013

TABLE 3

Genes and ESTs with Significant Age-Dependent Changes in Expression Level (ANOVA: $p \leq .05$) That Did Not Appear in TABLES 1 and 2

GenBank	Descriptions	Young	Mid	Age	FC	ANOVA p
Genes, Decreased						
	Correlate with both OMT and SWM					
M93273	somatostatin receptor subtype 2	1338 ± 142	1395 ± 105	1016 ± 30	-1.32	0.0252
AI175973	ESTs, Highly similar to NADH dehydrogenase	157 ± 18	136 ± 16	95 ± 14	-1.64	0.0314
AA799724	ESTs, Highly similar to DNA-directed RNA polymeraseI	2375 ± 47	2384 ± 79	2120 ± 91	-1.12	0.0321
X06769	FBJ v-fos oncogene homolog	1672 ± 156	1340 ± 154	1145 ± 79	-1.46	0.0329
X89696	TPCR06 protein	763 ± 50	625 ± 38	620 ± 35	-1.23	0.0361
D29766	v-crk-associated tyrosine kinase substrate	2478 ± 129	1929 ± 256	1568 ± 269	-1.58	0.0362
AI102839	cerebellar Ca-binding protein, spot 35 protein	2552 ± 110	2321 ± 131	2088 ± 110	-1.22	0.0364
M80550	adenylyl cyclase	6464 ± 207	6010 ± 212	5752 ± 133	-1.12	0.0403
U18771	Ras-related protein Rab-26	2631 ± 67	2373 ± 101	2350 ± 66	-1.12	0.0410
M36453	Inhibin, alpha	1438 ± 74	1350 ± 73	1178 ± 64	-1.22	0.0449
	Correlate with OMT					
AF055477	L-type voltage-dependent Ca^{2+} channel (α1D subunit)	2917 ± 144	2688 ± 119	2449 ± 74	-1.19	0.0275
AI013627	defender against cell death 1	10148 ± 175	9237 ± 310	9312 ± 219	-1.09	0.0289
AA891916	membrane interacting protein of RGS16	4586 ± 148	4330 ± 114	4117 ± 81	-1.11	0.0295
X67805	Synaptonemal complex protein 1	242 ± 22	189 ± 28	145 ± 23	-1.67	0.0319
D10874	lysosomal vacuolar proton pump (16 kDa)	23958 ± 745	21491 ± 849	21100 ± 812	-1.14	0.0436
D45247	proteasome subunit RCX	13926 ± 267	13333 ± 391	12526 ± 432	-1.11	0.0477
AF040954	putative protein phosphatase1 nuclear targeting subunit	1258 ± 27	1173 ± 35	1149 ± 28	-1.09	0.0515

TABLE 3

Genes and ESTs with Significant Age-Dependent Changes in Expression Level (ANOVA; $p \leq .05$) That Did Not Appear in TABLES 1 and 2

GenBank	Descriptions	Young	Mid	Age	FC	ANOVA p
Genes, Decreased						
	Correlate with SWM					
D10262	choline kinase	1248 ± 62	1092 ± 44	1079 ± 33	-1.16	0.0345
AI178921	Insulin degrading enzyme	174 ± 24	163 ± 9	111 ± 17	-1.56	0.0376
L29573	neurotransmitter transporter,noradrenalin	455 ± 47	342 ± 23	344 ± 31	-1.32	0.0475
	No significant behavioral correlations					
U75405	procollagen, type I, alpha 1	490 ± 18	378 ± 34	346 ± 22	-1.42	0.0017
L26292	Kruppel-like factor 4 (gut)	173 ± 21	100 ± 13	95 ± 10	-1.83	0.0018
AI169265	Atp6s1	18405 ± 380	16537 ± 447	16547 ± 318	-1.11	0.0027
L13202	RATHFH2 HNF-3/fork-head homolog-2 (HFH-2)	799 ± 63	557 ± 71	512 ± 19	-1.56	0.0027
AA799779	acyl-CoA:dihydroxyacetonephosphate acyltransferase	2742 ± 82	2363 ± 122	2181 ± 100	-1.26	0.0030
D89340	dipeptidylpeptidase III	2158 ± 76	1824 ± 68	1848 ± 64	-1.17	0.0038
AF019974	Chromogranin B, parathyroid secretory protein	10172 ± 290	8502 ± 400	8604 ± 334	-1.18	0.0038
U72620	Lot1	760 ± 52	620 ± 54	511 ± 35	-1.49	0.0042
U17254	immediate early gene transcription factor NGFI-B	3291 ± 202	2559 ± 115	2496 ± 180	-1.32	0.0045
M83745	Protein convertase subtilisin / kexin, type I	815 ± 43	630 ± 58	578 ± 39	-1.41	0.0048
AA893708	KIAA0560	2575 ± 62	2328 ± 84	2203 ± 74	-1.17	0.0061
H33725	associated molecule with the SH3 domain of STAM	1102 ± 26	970 ± 32	943 ± 41	-1.17	0.0064
AI230914	farnesyltransferase beta subunit	4044 ± 97	3465 ± 130	3498 ± 148	-1.16	0.0065
D37951	MIBP1 (c-myc intron binding protein 1)	6374 ± 194	5826 ± 173	5601 ± 100	-1.14	0.0067
AF076183	cytosolic sorting protein PACS-1a (PACS-1)	5098 ± 314	4039 ± 263	3774 ± 269	-1.35	0.0072
X82445	nuclear distribution gene C homolog (*Aspergillus*)	3311 ± 111	2910 ± 85	2901 ± 87	-1.14	0.0072
AA800948	Tuba4	8512 ± 215	7857 ± 402	6875 ± 342	-1.24	0.0076
D10699	ubiquitin carboxy-terminal hydrolase L1	19927 ± 1108	16996 ± 631	16532 ± 478	-1.21	0.0090
X57281	Glycine receptor alpha 2 subunit	199 ± 28	118 ± 19	111 ± 13	-1.79	0.0096
X76985	latexin	3937 ± 114	3187 ± 165	3332 ± 201	-1.18	0.0105
X84039	lumican	398 ± 30	283 ± 15	281 ± 36	-1.42	0.0109
U89905	alpha-methylacyl-CoA racemase	927 ± 39	793 ± 33	793 ± 27	-1.17	0.0110
M24852	Neuron specific protein PEP-19 (Purkinje cell protein 4)	6759 ± 349	5578 ± 280	5483 ± 310	-1.23	0.0146
U75917	clathrin-associated protein 17	6585 ± 232	5368 ± 330	5557 ± 291	-1.18	0.0158
X53427	glycogen synthase kinase 3 alpha (EC 2.7.1.37)	9799 ± 148	8843 ± 366	8572 ± 281	-1.14	0.0161
U28938	receptor-type protein tyrosine phosphatase D30	1564 ± 91	1354 ± 50	1286 ± 51	-1.22	0.0163
AA891880	Loc65042	2931 ± 59	2607 ± 85	2607 ± 98	-1.12	0.0171
AI232268	LDL receptor-related protein associated protein 1	1708 ± 68	1504 ± 59	1493 ± 36	-1.14	0.0186
AI045249	heat shock 70kD protein 8	537 ± 42	467 ± 46	366 ± 29	-1.47	0.0195
AF095927	protein phosphatase 2C	2968 ± 120	2516 ± 91	2549 ± 132	-1.16	0.0197
AA819708	Cox7a3	18590 ± 404	17401 ± 452	16742 ± 433	-1.11	0.0201
AA866257	ESTs	4750 ± 198	3994 ± 261	4021 ± 99	-1.18	0.0205
AA942685	cytosolic cysteine dioxygenase 1	9391 ± 397	8145 ± 443	7797 ± 325	-1.20	0.0221
D16478	mitochondrial long-chain enoyl-CoA hydratase	3913 ± 78	3615 ± 95	3499 ± 118	-1.12	0.0222
D88586	eosinophil cationic protein	2522 ± 108	2236 ± 206	1853 ± 138	-1.36	0.0226

TABLE 3

Genes and ESTs with Significant Age-Dependent Changes in Expression Level (ANOVA; $p \leq .05$) That Did Not Appear in TABLES 1 and 2

GenBank	Descriptions	Young	Mid	Age	FC	ANOVA p
Genes, Decreased						
	No significant behavioral correlations					
E03229	cytosolic cysteine dioxygenase 1	5634 ± 433	4518 ± 512	3918 ± 238	-1.44	0.0227
AB006451	Tim23	5968 ± 155	5562 ± 198	5315 ± 100	-1.12	0.0241
M10068	NADPH-cytochrome P-450 oxidoreductase	5771 ± 205	4998 ± 190	5139 ± 191	-1.12	0.0242
Z48225	protein synthesis initiation factor eIF-2B delta subunit	2710 ± 114	2415 ± 96	2327 ± 78	-1.16	0.0260
M93669	Secretogranin II	4917 ± 225	4395 ± 136	4309 ± 105	-1.14	0.0266
U17254	immediate early gene transcription factor NGFI-B	6004 ± 635	4395 ± 228	4694 ± 316	-1.28	0.0269
U38801	DNA polymerase beta	1173 ± 61	1001 ± 45	997 ± 39	-1.18	0.0270
AA874874	ESTs, Highly similar to alcohol dehydrogenase class III	3683 ± 64	3429 ± 83	3436 ± 60	-1.07	0.0278
AB016532	period homolog 2 (Drosophila)	1440 ± 117	1116 ± 84	1135 ± 62	-1.27	0.0290
AF007758	synuclein, alpha	17737 ± 473	15958 ± 751	15463 ± 459	-1.15	0.0295
U04738	Somatostatin receptor subtype 4	2066 ± 109	1680 ± 70	1733 ± 122	-1.19	0.0300
AF007890	resection-induced TPI (rsl1)	513 ± 48	388 ± 43	326 ± 50	-1.58	0.0307
AA874969	ESTs, Highly similar to c-Jun leucine zipper interactive	8555 ± 211	7333 ± 326	7531 ± 387	-1.14	0.0310
M31174	thyroid hormone receptor alpha	16273 ± 775	14217 ± 473	14395 ± 419	-1.13	0.0312
AA801286	Inositol (myo)-1(or 4)-monophosphatase 1	4767 ± 151	4270 ± 199	4155 ± 118	-1.15	0.0312
AF007554	Mucin1	385 ± 29	276 ± 35	282 ± 26	-1.37	0.0316
X98399	solute carrier family 14, member 1	2002 ± 105	1555 ± 95	1615 ± 151	-1.24	0.0329
AI168942	branched chain keto acid dehydrogenase E1	1580 ± 73	1367 ± 58	1418 ± 30	-1.11	0.0334
AF023087	Early growth response 1	20068 ± 1720	16426 ± 661	16294 ± 622	-1.23	0.0339
K02248	Somatostatin	4314 ± 165	3565 ± 189	3651 ± 245	-1.18	0.0341
AA859954	Vacuole Membrane Protein 1	4197 ± 122	3755 ± 119	3789 ± 128	-1.11	0.0346
AI176621	iron-responsive element-binding protein	1505 ± 66	1334 ± 63	1287 ± 42	-1.17	0.0348
AI010110	SH3-domain GRB2-like 1	1981 ± 67	1596 ± 113	1669 ± 117	-1.19	0.0363
L42855	transcription elongation factor B (SIII) polypeptide 2	10836 ± 201	9654 ± 417	9859 ± 283	-1.10	0.0368
AI136891	zinc finger protein 36, C3H type-like 1	3892 ± 153	3427 ± 188	3247 ± 160	-1.20	0.0369
S77492	Bone morphogenetic protein 3	123 ± 15	103 ± 17	65 ± 14	-1.89	0.0374
AI230778	ESTs, Highly similar to protein-tyrosine sulfotrans. 2	2049 ± 41	2019 ± 120	1714 ± 101	-1.20	0.0380
AA859980	T-complex 1	1710 ± 77	1411 ± 71	1478 ± 90	-1.16	0.0383
U27518	UDP-glucuronosyltransferase	316 ± 22	266 ± 26	223 ± 24	-1.42	0.0394
AF030088	RuvB-like protein 1	497 ± 151	252 ± 39	181 ± 21	-2.74	0.0398
AF013144	MAP-kinase phosphatase (cpg21)	1551 ± 185	1100 ± 98	1149 ± 92	-1.35	0.0408
M58404	thymosin, beta 10	20359 ± 853	18136 ± 773	17948 ± 400	-1.13	0.0413
AA819500	ESTs, Highly similar to AC12_HUMAN 37 kD subunit	532 ± 44	434 ± 30	411 ± 26	-1.29	0.0417
AF020046	integrin alpha E1, epithelial-associated	113 ± 17	109 ± 12	70 ± 10	-1.62	0.0419
D10854	aldehyde reductase	18091 ± 526	16744 ± 433	16538 ± 354	-1.09	0.0422
AF000899	p58/p45, nucleolin	1666 ± 114	1381 ± 81	1359 ± 73	-1.23	0.0430
S77858	non-muscle myosin alkali light chain	10848 ± 292	9865 ± 409	9642 ± 278	-1.12	0.0435
J05031	Isovaleryl Coenzyme A dehydrogenase	1996 ± 57	1799 ± 75	1792 ± 45	-1.11	0.0451

TABLE 3

Genes and ESTs with Significant Age-Dependent Changes in Expression Level (ANOVA; $p \leq .05$) That Did Not Appear in TABLES 1 and 2

GenBank	Descriptions	Young	Mid	Age	FC	ANOVA p
Genes, Decreased						
	No significant behavioral correlations					
J02773	heart fatty acid binding protein	2242 ± 88	1918 ± 118	1885 ± 99	-1.19	0.0453
AA891041	jun B proto-oncogene	1125 ± 128	788 ± 79	871 ± 68	-1.29	0.0453
AA817887	profilin	12549 ± 398	10859 ± 592	10886 ± 498	-1.15	0.0460
U38379	Gamma-glutamyl hydrolase	2340 ± 215	2136 ± 177	1693 ± 141	-1.38	0.0467
D78308	calreticulin	8256 ± 349	7233 ± 343	7446 ± 126	-1.11	0.0486
AA818487	cyclophilin B	8861 ± 410	7912 ± 293	7779 ± 236	-1.14	0.0488
AA799479	ESTs, Highly similar to NADH-ubiquinone oxidoreduct.	4937 ± 203	4124 ± 291	4075 ± 263	-1.21	0.0496
AI104388	heat shock 27kD protein 1	2102 ± 72	2072 ± 81	1839 ± 82	-1.14	0.0511
X59737	ubiquitous mitochondrial creatine kinase	11016 ± 315	9658 ± 360	9950 ± 451	-1.11	0.0512
D83948	adult liver S1-1 protein	1411 ± 45	1249 ± 78	1221 ± 30	-1.16	0.0522
AA893788	ESTs, Highly similar to chromobox protein homolog 5	658 ± 33	562 ± 23	568 ± 31	-1.16	0.0541
Genes, Increased						
	Correlate with both OMT and SWM					
AI230247	selenoprotein P, plasma, 1	7467 ± 279	8179 ± 312	8700 ± 319	1.17	0.0304
AF016269	kallikrein 6 (neurosin, zyme)	1141 ± 75	1166 ± 51	1375 ± 72	1.21	0.0353
AF021935	Ser-Thr protein kinase	2 ± 111	453 ± 193	649 ± 184	10.63	0.0395
M24104	synaptobrevin 2	1145 ± 55	1783 ± 260	1794 ± 210	1.57	0.0544
	Correlate with OMT					
AI235344	geranylgeranyltransferase type I (GGTase-I)	336 ± 21	362 ± 16	413 ± 21	1.23	0.0310
X60212	ASI homolog of bacterial ribosomal subunit protein L22	17230 ± 994	18514 ± 1115	21606 ± 1305	1.25	0.0365
U14950	tumor suppressor homolog (synapse associ. protein)	315 ± 29	507 ± 61	498 ± 64	1.58	0.0379
X53504	ribosomal protein L12	9290 ± 179	9922 ± 247	10210 ± 290	1.10	0.0448
AA955388	Na^+K^+ transporting ATPase 2, beta polypeptide 2	2361 ± 155	2863 ± 320	3237 ± 170	1.37	0.0451
X76489	CD9 cell surface glycoprotein	2485 ± 199	2713 ± 135	3106 ± 170	1.25	0.0467
D28110	myelin-associated oligodendrocytic basic protein	5947 ± 490	7855 ± 539	8814 ± 1109	1.48	0.0499
	Correlate with SWM					
U10357	pyruvate dehydrogenase kinase 2 subunit p45 (PDK2)	3565 ± 133	3921 ± 274	4485 ± 240	1.26	0.0292
D00569	2,4-dienoyl CoA reductase 1, mitochondrial	200 ± 22	241 ± 32	307 ± 24	1.54	0.0293
AA818240	Nuclear pore complex protein	308 ± 35	440 ± 42	424 ± 28	1.38	0.0329
M24104	synaptobrevin 2	685 ± 193	1379 ± 247	1581 ± 250	2.31	0.0332
D28557	cold shock domain protein A	1383 ± 89	1491 ± 129	1803 ± 106	1.30	0.0337
X54467	cathepsin D	3715 ± 294	4091 ± 388	5138 ± 431	1.38	0.0373
X13905	ras-related rab1B protein	201 ± 111	803 ± 179	689 ± 181	3.43	0.0388
AI228548	ESTs, Highly similar to DKFZp586G0322.1	1909 ± 140	2053 ± 75	2321 ± 110	1.22	0.0412
V01244	Prolactin	75 ± 37	70 ± 37	354 ± 140	4.75	0.0476
L24896	glutathione peroxidase 4	12303 ± 650	12725 ± 456	14045 ± 358	1.14	0.0479

TABLE 3

Genes and ESTs with Significant Age-Dependent Changes in Expression Level (ANOVA; $p \leq .05$) That Did Not Appear in TABLES 1 and 2

GenBank	Descriptions	Young	Mid	Age	FC	ANOVA p
Genes, Increased						
	No significant behavioral correlations					
U77777	interleukin 18	252 ± 15	290 ± 12	371 ± 32	1.47	0.0025
AI102299	Bid3	267 ± 98	527 ± 59	603 ± 21	2.26	0.0032
L19998	Phenol-preferring sulfotransferase 1A	373 ± 36	507 ± 27	616 ± 69	1.65	0.0065
AF051561	solute carrier family 12, member 2	2749 ± 82	3228 ± 83	3281 ± 163	1.19	0.0074
U08259	Glutamate receptor, N-methyl D-aspartate 2C	919 ± 34	989 ± 49	1118 ± 38	1.22	0.0074
AB008538	HB2	3733 ± 133	4436 ± 189	4264 ± 117	1.14	0.0087
AF016296	neuropilin	1838 ± 121	2279 ± 85	2259 ± 110	1.23	0.0111
X62950	pBUS30 with repetitive elements	360 ± 25	577 ± 67	548 ± 47	1.52	0.0124
AF030050	replication factor C	857 ± 62	1154 ± 73	1148 ± 81	1.34	0.0127
AA848831	lysophosphatidic acid G-protein-coupled receptor, 2	1854 ± 170	2729 ± 225	2784 ± 261	1.50	0.0129
M91234	VL30 element	2573 ± 152	3409 ± 221	3467 ± 254	1.35	0.0134
J05132	UDP-glucuronosyltransferase	968 ± 76	1283 ± 68	1212 ± 74	1.25	0.0148
AF008554	implantation-associated protein (IAG2)	362 ± 46	528 ± 33	500 ± 40	1.38	0.0162
AI231807	ferritin light chain 1	5496 ± 174	5863 ± 273	6469 ± 197	1.18	0.0163
S72594	tissue inhibitor of metalloproteinase 2	3615 ± 205	4386 ± 216	4227 ± 114	1.17	0.0170
S61868	Ryudocan/syndecan 4	6117 ± 292	6315 ± 211	7348 ± 385	1.20	0.0182
X06916	S100 calcium-binding protein A4	572 ± 40	630 ± 60	868 ± 99	1.52	0.0184
U67136	A kinase (PRKA) anchor protein 5	306 ± 59	531 ± 66	551 ± 61	1.80	0.0191
Y17295	thiol-specific antioxidant protein (1-Cys peroxiredoxin)	2414 ± 154	3037 ± 133	2998 ± 193	1.24	0.0221
D45249	protease (prosome, macropain) 28 subunit, alpha	4169 ± 119	4657 ± 205	4808 ± 121	1.15	0.0223
U67137	guanylate kinase associated protein	3198 ± 366	4262 ± 333	4338 ± 177	1.36	0.0229
AF074608	MHC class I antigen (RT1.EC2) gene	782 ± 129	940 ± 110	1213 ± 69	1.55	0.0231
U67080	r-MyT13	-29 ± 17	74 ± 38	92 ± 32	1.50	0.0250
AI013861	3-hydroxyisobutyrate dehydrogenase	3347 ± 136	3759 ± 101	3678 ± 73	1.10	0.0255
S53527	S100 calcium-binding protein, beta (neural)	25683 ± 925	25830 ± 765	29195 ± 1184	1.14	0.0266
D89730	Fibulin 3, fibulin-like extracellular matrix protein 1	239 ± 23	351 ± 52	424 ± 50	1.78	0.0271
D90211	Lysosomal-associated membrane protein 2	3095 ± 142	3577 ± 157	3715 ± 168	1.20	0.0276
AA859645	attractin	2647 ± 81	2871 ± 82	2942 ± 60	1.11	0.0278
X55153	ribosomal protein P2	18829 ± 779	19676 ± 485	21368 ± 641	1.13	0.0284
M55015	nucleolin	6685 ± 139	6738 ± 263	7385 ± 147	1.10	0.0297
L25605	Dynamin 2	759 ± 84	780 ± 71	1109 ± 129	1.46	0.0303
AI231807	ferritin light chain 1	9399 ± 508	10459 ± 538	11268 ± 329	1.20	0.0312
L00191	Fibronectin 1	395 ± 23	530 ± 44	557 ± 53	1.41	0.0316
D28110	myelin-associated oligodendrocytic basic protein	837 ± 127	1177 ± 106	1331 ± 141	1.59	0.0320
AI176595	Cathepsin L	2414 ± 73	2639 ± 57	2678 ± 80	1.11	0.0324
X14323	Fc receptor, IgG, alpha chain transporter	431 ± 38	510 ± 71	640 ± 42	1.49	0.0328
X74226	LL5 protein	2042 ± 69	2000 ± 66	2279 ± 92	1.12	0.0330
AA892775	Lysozyme	1760 ± 88	1781 ± 65	2438 ± 314	1.39	0.0337
X02904	glutathione S-transferase P subunit	2861 ± 124	3514 ± 276	3570 ± 141	1.25	0.0339
AI012589	glutathione S-transferase, pi 2	6325 ± 340	7706 ± 465	7807 ± 418	1.23	0.0353
AB000778	Phoshpolipase D gene 1	194 ± 24	270 ± 18	287 ± 31	1.48	0.0374

TABLE 3

Genes and ESTs with Significant Age-Dependent Changes in Expression Level (ANOVA; $p \leq .05$) That Did Not Appear in TABLES 1 and 2

GenBank	Descriptions	Young	Mid	Age	FC	ANOVA p
Genes, Increased						
	No significant behavioral correlations					
X97443	integral membrane protein Tmp21-I (p23)	862 ± 64	1194 ± 131	1211 ± 90	1.40	0.0396
X58294	carbonic anhydrase 2	5372 ± 252	6554 ± 399	6347 ± 290	1.18	0.0398
M99485	Myelin oligodendrocyte glycoprotein	2546 ± 107	2645 ± 113	3176 ± 259	1.25	0.0405
M23601	Monoamine oxidase B	4962 ± 268	5244 ± 152	5763 ± 212	1.16	0.0406
J05022	peptidylarginine deiminase	3834 ± 133	4231 ± 137	4503 ± 231	1.17	0.0425
Z49858	plasmolipin	2111 ± 146	2437 ± 69	2624 ± 172	1.24	0.0429
D17309	delta 4-3-ketosteroid-5-beta-reductase	568 ± 66	930 ± 96	951 ± 150	1.67	0.0432
AA955306	ras-related protein rab10	3912 ± 289	4796 ± 339	4975 ± 257	1.27	0.0444
M19936	Prosaposin- sphingolipid hydrolase activator	12981 ± 997	14182 ± 780	16095 ± 751	1.24	0.0463
M57276	Leukocyte antigen (Ox-44)	879 ± 79	1071 ± 65	1117 ± 57	1.27	0.0469
J02752	acyl-coA oxidase	1853 ± 119	2187 ± 155	2344 ± 118	1.26	0.0470
U78517	cAMP-regulated guanine nucleotide exchange factor II	3400 ± 134.	3956 ± 216	3903 ± 113	1.15	0.0477
AI102031	myc box dependent interacting protein 1	6381 ± 242	6919 ± 237	7265 ± 236	1.14	0.0486
M89646	ribosomal protein S24	14041 ± 448	15044 ± 319	15482 ± 416	1.10	0.0491
AA924925	ER transmembrane protein Dri 42	435 ± 209	799 ± 143	1067 ± 160	2.45	0.0493
X16933	RNA binding protein p45AUF1	1516 ± 166	2186 ± 203	2139 ± 221	1.41	0.0499
X72757	cox VIa gene (liver)	666 ± 73	855 ± 39	829 ± 51	1.24	0.0502
AA957132	N-acetylglucosaminyltransferase I	242 ± 26	401 ± 56	398 ± 54	1.64	0.0508
AA818025	CD59 antigen	5668 ± 298	6175 ± 280	6909 ± 414	1.22	0.0509
AI237007	ESTs, Highly similar to flavoprot.-ubiquin. Oxidoreduct.	48 ± 37	117 ± 50	195 ± 29	3.19	0.0519
U07619	Coagulation factor III (thromboplastin, tissue factor)	701 ± 37	792 ± 37	847 ± 46	1.21	0.0544
ESTs, Decreased						
	Correlate with both OMT and SWM					
AA874830	UI-R-E0-cg-f-04-0-UI.s1 cDNA	1584 ± 87	1406 ± 65	1323 ± 33	-1.20	0.0268
AA875032	UI-R-E0-cb-h-09-0-UI.s1 cDNA	1770 ± 40	1536 ± 91	1490 ± 72	-1.19	0.0288
AA799599	EST189096 cDNA	6628 ± 210	6184 ± 281	5618 ± 257	-1.18	0.0328
AA892813	EST196616 cDNA	218 ± 41	241 ± 54	92 ± 25	-2.37	0.0363
AA799529	EST189026 cDNA	1590 ± 61	1529 ± 51	1388 ± 55	-1.15	0.0466
AA893584	EST197387 cDNA	4021 ± 120	3570 ± 206	3416 ± 167	-1.18	0.0548
	Correlate with OMT					
AA894305	EST198108 cDNA	4779 ± 107	4393 ± 138	4261 ± 151	-1.12	0.0349
AA800622	EST190119 cDNA	2372 ± 76	2325 ± 102	2056 ± 83	-1.15	0.0370
AA893690	EST197493 cDNA	5102 ± 229	4813 ± 146	4334 ± 220	-1.18	0.0378
AA891221	EST195024 cDNA	4562 ± 179	4159 ± 173	3956 ± 128	-1.15	0.0423
AA893320	EST197123 cDNA	1110 ± 35	1071 ± 69	911 ± 57	-1.22	0.0455
AA891537	EST195340 cDNA	2420 ± 94	2098 ± 85	2145 ± 96	-1.13	0.0468
AA799680	EST189177 cDNA	560 ± 45	544 ± 33	431 ± 39	-1.30	0.0504

TABLE 3

Genes and ESTs with Significant Age-Dependent Changes in Expression Level (ANOVA; $p \leq .05$) That Did Not Appear in TABLES 1 and 2

GenBank	Descriptions	Young	Mid	Age	FC	ANOVA p
ESTs, Decreased						
	Correlate with SWM					
AA893199	EST197002 cDNA	2422 ± 100	2482 ± 67	2129 ± 112	-1.14	0.0287
AA799636	EST189133 cDNA	3279 ± 92	2986 ± 125	2826 ± 124	-1.16	0.0358
AA874995	UI-R-E0-cf-d-08-0-UI.s1 cDNA	1202 ± 44	1123 ± 37	1068 ± 19	-1.13	0.0360
AA892298	EST196101 cDNA	302 ± 26	243 ± 13	229 ± 22	-1.32	0.0456
AA892538	EST196341 cDNA	1033 ± 64	902 ± 41	868 ± 36	-1.19	0.0547
	No significant behavioral correlations					
AA859690	UI-R-E0-bx-e-11-0-UI.s1 cDNA	297 ± 29	173 ± 40	137 ± 10	-2.17	0.0017
AA875004	UI-R-E0-cb-b-07-0-UI.s1 cDNA	965 ± 40	774 ± 44	776 ± 30	-1.24	0.0022
AA891037	EST194840 cDNA	2174 ± 98	1781 ± 83	1774 ± 68	-1.23	0.0031
AA893185	EST196988 cDNA	7616 ± 301	6680 ± 137	6666 ± 166	-1.14	0.0045
AA892511	EST196314 cDNA	4716 ± 113	4061 ± 150	4216 ± 139	-1.12	0.0068
AA875129	UI-R-E0-bu-e-01-0-UI.s2 cDNA	1214 ± 28	1093 ± 33	1062 ± 34	-1.14	0.0071
AA800693	EST190190 cDNA	3177 ± 84	2844 ± 82	2830 ± 71	-1.12	0.0072
AA859562	UI-R-E0-bv-b-03-0-UI.s1 cDNA	933 ± 91	682 ± 57	606 ± 58	-1.54	0.0078
AA860030	UI-R-E0-bz-e-07-0-UI.s2 cDNA	20727 ± 774	17601 ± 811	17941 ± 508	-1.16	0.0090
AA891727	EST195530 cDNA	5801 ± 266	4821 ± 204	5038 ± 189	-1.15	0.0114
AA892796	EST196599 cDNA	6952 ± 143	6326 ± 167	6441 ± 110	-1.08	0.0117
AI639477	mixed-tissue library cDNA clone rx02351 3	264 ± 26	193 ± 53	78 ± 40	-3.39	0.0154
AA893717	EST197520 cDNA	515 ± 24	442 ± 35	386 ± 27	-1.33	0.0179
AA892414	EST196217 cDNA	2935 ± 143	2507 ± 111	2511 ± 79	-1.17	0.0185
AA893743	EST197546 cDNA	2730 ± 120	2282 ± 121	2181 ± 154	-1.25	0.0193
AI176491	EST220076 cDNA	5180 ± 182	4665 ± 213	4450 ± 108	-1.16	0.0199
AA799481	EST188978 cDNA	1036 ± 33	889 ± 31	916 ± 44	-1.13	0.0240
AA859643	UI-R-E0-bs-a-08-0-UI.s1 cDNA	4772 ± 162	3978 ± 177	4165 ± 238	-1.15	0.0252
AA875257	UI-R-E0-cq-d-12-0-UI.s1 cDNA	1715 ± 133	1369 ± 92	1342 ± 71	-1.28	0.0255
AA685974	EST108806 cDNA	5543 ± 142	4855 ± 194	4974 ± 184	-1.11	0.0275
AA891476	EST195279 cDNA	7512 ± 289	7075 ± 235	6520 ± 208	-1.15	0.0279
AA891950	EST195753 cDNA	865 ± 18	818 ± 45	725 ± 33	-1.19	0.0284
AA875019	UI-R-E0-cb-f-08-0-UI.s1 cDNA	1007 ± 32	908 ± 29	901 ± 29	-1.12	0.0357
AA866477	UI-R-E0-br-h-03-0-UI.s1 cDNA	11037 ± 230	9932 ± 341	10208 ± 283	-1.08	0.0376
AI639209	mixed-tissue library cDNA clone rx00680 3	763 ± 57	820 ± 98	562 ± 44	-1.36	0.0385
AI102868	EST212157 cDNA	11364 ± 316	9876 ± 516	9787 ± 490	-1.16	0.0418
AI178204	EST221869 cDNA	2465 ± 180	2162 ± 137	1905 ± 122	-1.29	0.0419
AA799858	EST189355 cDNA	1068 ± 76	925 ± 58	827 ± 58	-1.29	0.0427
AA800026	EST189523 cDNA	249 ± 29	155 ± 26	144 ± 35	-1.73	0.0429
AA892637	EST196440 cDNA	809 ± 16	757 ± 24	739 ± 16	-1.10	0.0430
AA859545	ESTs, Weakly similar to hypothetical protein C09H6.3	3289 ± 167	2762 ± 137	2876 ± 134	-1.14	0.0442
AA859848	UI-R-E0-cc-h-10-0-UI.s1 cDNA	3396 ± 315	3150 ± 165	2626 ± 129	-1.29	0.0456
H33086	EST108750 cDNA	21205 ± 763	18706 ± 530	19138 ± 810	-1.11	0.0477
AA893224	EST197027 cDNA	2325 ± 67	2150 ± 75	2076 ± 64	-1.12	0.0502

TABLE 3

Genes and ESTs with Significant Age-Dependent Changes in Expression Level (ANOVA; $p \leq .05$) That Did Not Appear in TABLES 1 and 2

GenBank	Descriptions	Young	Mid	Age	FC	ANOVA p
ESTs, Increased						
	Correlate with both OMT and SWM					
AA893946	EST197749 cDNA	371 ± 45	565 ± 43	544 ± 72	1.47	0.0440
	Correlate with OMT					
AI638997	mixed-tissue library cDNA clone rx05048 3	402 ± 23	450 ± 26	483 ± 11	1.20	0.0381
AI177404	EST221024 cDNA	1012 ± 46	1193 ± 73	1245 ± 65	1.23	0.0429
	Correlate with SWM					
AA800318	EST189815 cDNA	315 ± 46	376 ± 40	474 ± 41	1.51	0.0421
	No significant behavioral correlations					
AA893082	EST196885 cDNA	1454 ± 95	1902 ± 43	1865 ± 110	1.28	0.0021
AA892986	EST196789 cDNA	586 ± 19	627 ± 33	756 ± 39	1.29	0.0025
M13100	long interspersed repetitive DNA sequence LINE3	4328 ± 230	5963 ± 252	5947 ± 457	1.37	0.0026
AA891734	EST195537 cDNA	1648 ± 86	1778 ± 82	2045 ± 60	1.24	0.0037
AI171966	ESTs, Highly similar to selenide, water dikinase 2	880 ± 42	934 ± 30	1181 ± 93	1.34	0.0049
AI639151	mixed-tissue library cDNA clone rx02802 3	939 ± 49	1192 ± 80	1223 ± 54	1.30	0.0083
AA875037	UI-R-E0-cb-a-03-0-UI.s1 cDNA	11 ± 71	268 ± 70	357 ± 78	5.84	0.0084
AA891690	ESTs, Weakly similar to p-serine aminotransferase	1858 ± 76	1955 ± 65	2296 ± 131	1.24	0.0088
AA891810	EST195613 cDNA	1504 ± 140	2028 ± 155	2274 ± 202	1.51	0.0125
AA866432	UI-R-E0-ch-e-06-0-UI.s1 cDNA	2777 ± 150	3380 ± 102	3493 ± 226	1.26	0.0143
X05472	2.4 kb repeat DNA right terminal region	4188 ± 565	5325 ± 564	7241 ± 899	1.73	0.0173
AA892146	EST195949 cDNA	5386 ± 450	7073 ± 436	7004 ± 418	1.30	0.0187
AA852046	EST194815 cDNA	1697 ± 140	2163 ± 92	2051 ± 112	1.21	0.0234
AA799396	EST188893 cDNA	163 ± 26	264 ± 35	269 ± 24	1.65	0.0275
AI638971	mixed-tissue library cDNA clone rx04989 3	128 ± 26	188 ± 13	213 ± 24	1.67	0.0285
AA892520	EST196323 cDNA	479 ± 31	526 ± 28	601 ± 33	1.25	0.0305
AA891774	EST195577 cDNA	-518 ± 92	-115 ± 126	-147 ± 108	1.00	0.0322
M13100	long interspersed repetitive DNA sequence LINE3	8845 ± 982	12115 ± 1117	12282 ± 814	1.39	0.0366
AI639257	mixed-tissue library cDNA clone rx01119 3	172 ± 23	306 ± 41	286 ± 41	1.66	0.0386
AA866299	UI-R-A0-ac-f-12-0-UI.s3 cDNA	684 ± 45	810 ± 24	885 ± 73	1.29	0.0390
AA799773	EST189270 cDNA	299 ± 30	408 ± 24	433 ± 50	1.45	0.0407
AA866299	UI-R-A0-ac-f-12-0-UI.s3 cDNA	522 ± 32	623 ± 28	626 ± 31	1.20	0.0415
AA891944	EST195747 cDNA	193 ± 15	198 ± 13	247 ± 20	1.28	0.0488

[106] Using the method of the invention, we have identified a set of genes and ESTs that changed with age by ANOVA ($p \leq .05$), but which are not ACGs. These include AA685974 (EST108806 cDNA); AA799396 (EST188893 cDNA); AA799479 (ESTs, Highly similar to NADH-ubiquinone oxidoreduct.); AA799481 (EST188978 cDNA); AA799529 (EST189026 cDNA); AA799599 (EST189096 cDNA); AA799636 (EST189133 cDNA); AA799680 (EST189177 cDNA); AA799724 (ESTs, Highly similar to DNA-directed RNA polymeraseI); AA799773 (EST189270 cDNA); AA799779 (acyl-CoA:dihydroxyacetonephosphate acyltransferase); AA799858 (EST189355 cDNA); AA800026 (EST189523 cDNA); AA800318 (EST189815 cDNA); AA800622 (EST190119 cDNA); AA800693 (EST190190 cDNA); AA800948 (Tuba4); AA801286 (Inositol (myo)-1(or 4)-monophosphatase 1); AA817887 (profilin); AA818025 (CD59 antigen); AA818240 (Nuclear pore complex

protein); AA818487 (cyclophilin B); AA819500 (ESTs, Highly similar to AC12_HUMAN 37 kD subunit); AA819708 (Cox7a3); AA848831 (lysophosphatidic acid G-protein-coupled receptor, 2); AA852046 (EST194815 cDNA); AA859545 (ESTs, Weakly similar to hypothetical protein C09H6.3); AA859562 (UI-R-E0-bv-b-03-0-UI.s1 cDNA); AA859643 (UI-R-E0-bs-a-08-0-UI.s1 cDNA); AA859645 (attractin); AA859690 (UI-R-E0-bx-e-11-0-UI.s1 cDNA); AA859848 (UI-R-E0-cc-h-10-0-UI.s1 cDNA); AA859954 (Vacuole Membrane Protein 1); AA859980 (T-complex 1); AA860030 (UI-R-E0-bz-e-07-0-UI.s2 cDNA); AA866257 (ESTs); AA866299 (UI-R-A0-ac-f-12-0-UI.s3 cDNA); AA866299 (UI-R-A0-ac-f-12-0-UI.s3 cDNA); AA866432 (UI-R-E0-ch-e-06-0-UI.s1 cDNA); AA866477 (UI-R-E0-br-h-03-0-UI.s1 cDNA); AA874830 (UI-R-E0-cg-f-04-0-UI.s1 cDNA); AA874874 (ESTs, Highly similar to alcohol dehydrogenase class III); AA874969 (ESTs, Highly similar to c-Jun leucine zipper interactive); AA874995 (UI-R-E0-cf-d-08-0-UI.s1 cDNA); AA875004 (UI-R-E0-cb-b-07-0-UI.s1 cDNA); AA875019 (UI-R-E0-cb-f-08-0-UI.s1 cDNA); AA875032 (UI-R-E0-cb-h-09-0-UI.s1 cDNA); AA875037 (UI-R-E0-cb-a-03-0-UI.s1 cDNA); AA875129 (UI-R-E0-bu-e-01-0-UI.s2 cDNA); AA875257 (UI-R-E0-cq-d-12-0-UI.s1 cDNA); AA891037 (EST194840 cDNA); AA891041 (jun B proto-oncogene); AA891221 (EST195024 cDNA); AA891476 (EST195279 cDNA); AA891537 (EST195340 cDNA); AA891690 (ESTs, Weakly similar to p-serine aminotransferase); AA891727 (EST195530 cDNA); AA891734 (EST195537 cDNA); AA891774 (EST195577 cDNA); AA891810 (EST195613 cDNA); AA891880 (Loc65042); AA891916 (membrane interacting protein of RGS16); AA891944 (EST195747 cDNA); AA891950 (EST195753 cDNA); AA892146 (EST195949 cDNA); AA892298 (EST196101 cDNA); AA892414 (EST196217 cDNA); AA892511 (EST196314 cDNA); AA892520 (EST196323 cDNA); AA892538 (EST196341 cDNA); AA892637 (EST196440 cDNA); AA892775 (Lysozyme); AA892796 (EST196599 cDNA); AA892813 (EST196616 cDNA); AA892986 (EST196789 cDNA); AA893082 (EST196885 cDNA); AA893185 (EST196988 cDNA); AA893199 (EST197002 cDNA); AA893224 (EST197027 cDNA); AA893320 (EST197123 cDNA); AA893584 (EST197387 cDNA); AA893690 (EST197493 cDNA); AA893708 (KIAA0560); AA893717 (EST197520 cDNA); AA893743 (EST197546 cDNA); AA893788 (ESTs, Highly similar to chromobox protein homolog 5); AA893946 (EST197749 cDNA); AA894305 (EST198108 cDNA); AA924925 (ER transmembrane protein Dri 42); AA942685 (cytosolic cysteine dioxygenase 1); AA955306 (ras-related protein rab10); AA955388 (Na^+K^+ transporting

ATPase 2, beta polypeptide 2); AA957132 (N-acetylglucosaminyltransferase I); AB000778 (Phoshpolipase D gene 1); AB006451 (Tim23); AB008538 (HB2); AB016532 (period homolog 2 (Drosophila)); AF000899 (p58/p45, nucleolin); AF007554 (Mucin1); AF007758 (synuclein, alpha); AF007890 (resection-induced TPI (rs11)); AF008554 (implantation-associated protein (IAG2)); AF013144 (MAP-kinase phosphatase (cpg21)); AF016269 (kallikrein 6 (neurosin, zyme)); AF016296 (neuropilin); AF019974 (Chromogranin B, parathyroid secretory protein); AF020046 (integrin alpha E1, epithelial-associated); AF021935 (Ser-Thr protein kinase); AF023087 (Early growth response 1); AF030050 (replication factor C); AF030088 (RuvB-like protein 1); AF040954 (putative protein phosphatase1 nuclear targeting subunit); AF051561 (solute carrier family 12, member 2); AF055477 (L-type voltage-dependent Ca^{2+} channel (?1D subunit)); AF074608 (MHC class I antigen (RT1.EC2) gene); AF076183 (cytosolic sorting protein PACS-1a (PACS-1)); AF095927 (protein phosphatase 2C); AI010110 (SH3-domain GRB2-like 1); AI012589 (glutathione S-transferase, pi 2); AI013627 (defender against cell death 1); AI013861 (3-hydroxyisobutyrate dehydrogenase); AI045249 (heat shock 70kD protein 8); AI102031 (myc box dependent interacting protein 1); AI102299 (Bid3); AI102839 (cerebellar Ca-binding protein, spot 35 protein); AI102868 (EST212157 cDNA); AI104388 (heat shock 27kD protein 1); AI136891 (zinc finger protein 36, C3H type-like 1); AI168942 (branched chain keto acid dehydrogenase E1); AI169265 (Atp6s1); AI171966 (ESTs, Highly similar to selenide, water dikinase 2); AI175973 (ESTs, Highly similar to NADH dehydrogenase); AI176491 (EST220076 cDNA); AI176595 (Cathepsin L); AI176621 (iron-responsive element-binding protein); AI177404 (EST221024 cDNA); AI178204 (EST221869 cDNA); AI178921 (Insulin degrading enzyme); AI228548 (ESTs, Highly similar to DKFZp586G0322.1); AI230247 (selenoprotein P, plasma, 1); AI230778 (ESTs, Highly similar to protein-tyrosine sulfotrans. 2); AI230914 (farnesyltransferase beta subunit); AI231807 (ferritin light chain 1); AI231807 (ferritin light chain 1); AI232268 (LDL receptor-related protein associated protein 1); AI235344 (geranylgeranyltransferase type I (GGTase-I)); AI237007 (ESTs, Highly similar to flavoprot.-ubiquin. Oxidoreduct.); AI638971 (mixed-tissue library cDNA clone rx04989 3); AI638997 (mixed-tissue library cDNA clone rx05048 3); AI639151 (mixed-tissue library cDNA clone rx02802 3); AI639209 (mixed-tissue library cDNA clone rx00680 3); AI639257 (mixed-tissue library cDNA clone rx01119 3); AI639477 (mixed-tissue library cDNA clone rx02351 3); D00569 (2,4-dienoyl CoA reductase 1,

mitochondrial); D10262 (choline kinase); D10699 (ubiquitin carboxy-terminal hydrolase L1); D10854 (aldehyde reductase); D10874 (lysosomal vacuolar proton pump (16 kDa)); D16478 (mitochondrial long-chain enoyl-CoA hydratase); D17309 (delta 4-3-ketosteroid-5-beta-reductase); D28110 (myelin-associated oligodendrocytic basic protein); D28110 (myelin-associated oligodendrocytic basic protein); D28557 (cold shock domain protein A); D29766 (v-crk-associated tyrosine kinase substrate); D37951 (MIBP1 (c-myc intron binding protein 1)); D45247 (proteasome subunit RCX); D45249 (protease (prosome, macropain) 28 subunit, alpha); D78308 (calreticulin); D83948 (adult liver S1-1 protein); D88586 (eosinophil cationic protein); D89340 (dipeptidylpeptidase III); D89730 (Fibulin 3, fibulin-like extracellular matrix protein 1); D90211 (Lysosomal-associated membrane protein 2); E03229 (cytosolic cysteine dioxygenase 1); H33086 (EST108750 cDNA); H33725 (associated molecule with the SH3 domain of STAM); J02752 (acyl-coA oxidase); J02773 (heart fatty acid binding protein); J05022 (peptidylarginine deiminase); J05031 (Isovaleryl Coenzyme A dehydrogenase); J05132 (UDP-glucuronosyltransferase); K02248 (Somatostatin); L00191 (Fibronectin 1); L13202 (RATHFH2 HNF-3/fork-head homolog-2 (HFH-2)); L19998 (sulfotransferase family 1A, phenol-preferring, member 1); L24896 (glutathione peroxidase 4); L25605 (Dynamin 2); L26292 (Kruppel-like factor 4 (gut)); L29573 (neurotransmitter transporter,noradrenalin); L42855 (transcription elongation factor B (SIII) polypeptide 2); M10068 (NADPH-cytochrome P-450 oxidoreductase); M13100 (long interspersed repetitive DNA sequence LINE3); M13100 (long interspersed repetitive DNA sequence LINE3); M19936 (Prosaposin- sphingolipid hydrolase activator); M23601 (Monoamine oxidase B); M24104 (synaptobrevin 2); M24104 (Vesicle-associated membrane protein (synaptobrevin 2)); M24852 (Neuron specific protein PEP-19 (Purkinje cell protein 4)); M31174 (thyroid hormone receptor alpha); M36453 (Inhibin, alpha); M55015 (nucleolin); M57276 (Leukocyte antigen (Ox-44)); M58404 (thymosin, beta 10); M80550 (adenylyl cyclase); M83745 (Protein convertase subtilisin / kexin, type I); M89646 (ribosomal protein S24); M91234 (VL30 element); M93273 (somatostatin receptor subtype 2); M93669 (Secretogranin II); M99485 (Myelin oligodendrocyte glycoprotein); S53527 (S100 calcium-binding protein, beta (neural)); S61868 (Ryudocan/syndecan 4); S72594 (tissue inhibitor of metalloproteinase 2); S77492 (Bone morphogenetic protein 3); S77858 (non-muscle myosin alkali light chain); U04738 (Somatostatin receptor subtype 4); U07619 (Coagulation factor III (thromboplastin, tissue factor)); U08259 (Glutamate receptor, N-methyl D-aspartate 2C); U10357 (pyruvate

dehydrogenase kinase 2 subunit p45 (PDK2)); U14950 (tumor suppressor homolog (synapse associ. protein)); U17254 (immediate early gene transcription factor NGFI-B); U17254 (immediate early gene transcription factor NGFI-B); U18771 (Ras-related protein Rab-26); U27518 (UDP-glucuronosyltransferase); U28938 (receptor-type protein tyrosine phosphatase D30); U38379 (Gamma-glutamyl hydrolase); U38801 (DNA polymerase beta); U67080 (r-MyT13); U67136 (A kinase (PRKA) anchor protein 5); U67137 (guanylate kinase associated protein); U72620 (Lot1); U75405 (procollagen, type I, alpha 1); U75917 (clathrin-associated protein 17); U77777 (interleukin 18); U78517 (cAMP-regulated guanine nucleotide exchange factor II); U89905 (alpha-methylacyl-CoA racemase); V01244 (Prolactin); X02904 (glutathione S-transferase P subunit); X05472 (2.4 kb repeat DNA right terminal region); X06769 (FBJ v-fos oncogene homolog); X06916 (S100 calcium-binding protein A4); X13905 (ras-related rab1B protein); X14323 (Fc receptor, IgG, alpha chain transporter); X16933 (RNA binding protein p45AUF1); X53427 (glycogen synthase kinase 3 alpha (EC 2.7.1.37)); X53504 (ribosomal protein L12); X54467 (cathepsin D); X55153 (ribosomal protein P2); X57281 (Glycine receptor alpha 2 subunit); X58294 (carbonic anhydrase 2); X59737 (ubiquitous mitochondrial creatine kinase); X60212 (ASI homolog of bacterial ribosomal subunit protein L22); X62950 (pBUS30 with repetitive elements); X67805 (Synaptonemal complex protein 1); X72757 (cox VIa gene (liver)); X74226 (LL5 protein); X76489 (CD9 cell surface glycoprotein); X76985 (latexin); X82445 (nuclear distribution gene C homolog (*Aspergillus*)); X84039 (lumican); X89696 (TPCR06 protein); X97443 (integral membrane protein Tmp21-I (p23)); X98399 (solute carrier family 14, member 1); Y17295 (thiol-specific antioxidant protein (1-Cys peroxiredoxin)); Z48225 (protein synthesis initiation factor eIF-2B delta subunit); Z49858 (plasmolipin)

[107] Using the method of the invention, we have also identified a set of genes and ESTs that changed with age ($p \leq .05$), but which are correlated with cognitive performance in behavioral tests. These include L03294 (Lpl, lipoprotein lipase); M18416 (Egr1, Early growth response 1 (Krox-24)); S68245 (Ca4, carbonic anhydrase 4); M64780 (Agrn, Agrin); M27207 (Col1a1, Procollagen- type I (alpha 1)); X16554 (Prps1, Phosphoribosyl pyrophosphate synthetase 1); M92433 (NGFI-C, Zinc-finger transcription factor (early response gene)); AA859975 (LOC64201, 2-oxoglutarate carrier); L08595 (Nuclear receptor subfamily 4, group A, member 2); M24542 (RISP, Rieske iron-sulfur protein); AI030089 (Nopp130, nucleolar phosphoprotein p130); AF104362 (Omd, Osteomodulin (osteoadherin));

L46873 (Slc15a1, Oligopeptide transporter); AI176689 (MAPKK 6, mitogen-activated protein kinase kinase 6); U66470 (rCGR11, Cell growth regulator); AF016387 (RXRG, retinoid X-receptor gamma); M18467 (Got2, glutamate oxaloacetate transaminase 2); X54793 (Hsp60, heat shock protein 60); X64401 (Cyp3a3, Cytochrome P450- subfamily IIIA (polypeptide 3)); M37584 (H2afz, H2A histone family (member Z)); L21192, (GAP-43, membrane attached signal protein 2 (brain)); AA875047 (TCPZ, T-complex protein 1 (zeta subunit)); U90610 (Cxcr4, CXC chemokine receptor); AF003904 (CRH-binding protein); U83880 (GPDH-M, glycerol-3-phosphate dehydrogenase, mitochondrial); X89703 (TPCR19, Testis Polymerase Chain Reaction product 19); D63886 (MMP16, matrix metalloproteinase 16); J05499 (GLS, glutaminase (mitochondrial)); D21799 (Psmb2, Proteasome subunit (beta type 2)); AA800794 (HT2A, zinc-finger protein); U90887 (Arg2, arginase type II); S82649 (Narp, neuronal activity-regulated pentraxin); M74223 (VGF, neurosecretory protein); AA874794 (Bex3, brain expressed X-linked 3); M15191 (Tac1, Tachykinin); AA892506 (coronin, actin binding protein 1A); L04485 (MAPPK1, mitogen-activated protein kinase kinase 1); AA799641 (S164, Contains a PWI domain associated with RNA splicing); AA817892 (Gnb2, Guanine nucleotide binding protein (beta 2 subunit)); AA893939 (DSS1, deleted in split hand/ split foot protein 1); AF000901 (P58/P45, Nucleoporin p58); AF087037 (Btg3, B-cell translocation gene 3); AB000280 (PHT1, peptide/histidine transporter); M87854 (Beta-ARK-1, beta adrenergic receptor kinase 1); U06099 (Prdx2, Peroxiredoxin 2); AF058795 (Gb2, GABA-B receptor); AA800517 (VAP1, vesicle associated protein); U63740 (Fez1, Protein kinase C-binding protein Zeta1); U53922 (Hsj2, DnaJ-like protein (RDJ1)); U78102 (Egr2, Early growth response 2); U44948 (SmLIM, smooth muscle cell LIM protein); U87627 (MCT3, putative monocarboxylate transporter); AB020504 (PMF31, highly homologus to mouse F-box-WD40 repeat protein 6); M21354 (Col3a1, collagen type III alpha-1); AA893664 (Temo, sertoli cell marker (KIAA0077 protein fragment)); AB010437 (CDH8, Cadherin-8); M22756 (Ndufv2, mitochondrial NADH dehydrogenase (24 kDa)); AA799389 (Rab3B, ras-related protein); AI172476 (Tieg-1, TGF-beta-inducible early growth response protein 1); AF091563 (Olfactory receptor); M64376 (Olfactory protein); J04488 (Ptgds, Prostaglandin D synthase); X71127 (c1qb, complement component 1- q (beta polypeptide)); J03752 (Microsomal GST-1, glutathione S-transferase); J03481 (Qdpr, Dihydropteridine reductase); L40362 (MHC class I RT1.C-type protein); M94918 (Hbb, beta hemoglobin); M55534 (Cryab, alpha crystallin polypeptide 2); U17919

(Aif1, allograft inflammatory factor 1); M15562 (MHC class II RT1.u-D-alpha chain); AA799645 (Phospholemman, FXYD domain-containing ion transport regulator 1); X13044 (Cd74, CD74 antigen); M24324 (RTS, MHC class I RT1 (RTS) (u haplotype)); U31866 (Nclone10); M32062 (Fcgr3, Fc IgG receptor III (low affinity)); AF095741 (Mg87); L03201 (Ctss, cathepsin S); M27905 (Rpl21, Ribosomal protein L21); D38380 (Tf, Transferrin); AA893493 (RPL26, Ribosomal protein L26); AJ222813 (Il18, interleukin 18); E13541 (Cspg5, chondroitin sulfate proteoglycan 5); X54096 (Lcat, Lecithin-cholesterol acyltransferase); L40364 (RT1Aw2, RT1 class Ib); D28111 (MOBP, myelin-associated oligodendrocytic basic protein); M32016 (Lamp2, lysosomal-associated membrane protein 2); X13167 (NF1-A, nuclear factor 1 A); U26356 (S100A1, S100 protein (alpha chain)); AI231213 (Kangai 1, suppression of tumorigenicity 6); AI170268 (Ptgfr, Prostaglandin F receptor); X62952 (Vim, vimentin); AI014169 (Vdup1, vitamin D-upregulated); AA850219 (Anx3, Annexin A3); D84477 (Rhoa, ras-related homolog A2); X52477 (C3, Complement component 3); X52619 (Rpl28, Ribosomal protein L28); X06554 (S-MAG, myelin-associated glycoprotein C-term); Z50144 (Kat2, kynurenine aminotransferase II); X14181 (RPL18A, Ribosomal protein L18a); AA892333 (Tuba1, alpha-tubulin); U67082 (KZF-1, Kruppel associated box (KRAB) zinc finger 1); U11760 (Vcp, valosin-containing protein); AF048828 (VDAC1, voltage-dependent anion channel 1); M31076 (TNF-alpha, Transforming growth factor (alpha)); S83279 (HSDIV, 17-beta-hydroxysteroid dehydrogenase type IV); AI102103 (Pik4cb, Phosphatidylinositol 4-kinase); X56325 (Hba1, alpha 1 hemoglobin); X73371 (FCGR2, Low affinity immunoglobulin gamma Fc receptor II); X78848 (Gsta1, Glutathione-S-transferase (alpha type)); U92564 (Roaz, Olf-1/EBF associated Zn finger protein); AI171462 (Cd24, CD24 antigen); X83231 (PAIHC3, Pre-alpha-inhibitor, heavy chain 3); AF097593 (Ca4, cadherin 2- type 1 (neuronal)); X68283 (Rpl29, Ribosomal protein L29); S55427 (Pmp, peripheral myelin protein); AA818025 (Cd59, CD59 antigen); E01534 (Rps15, Ribosomal protein S15); U37138 (Sts, Steroid sulfatase); X55572 (Apod, Apolipoprotein D); AI028975 (AP-1, adaptor protein complex (beta 1)); L16995 (ADD1, adipocyte determination/ differentiation-dependent factor 1); U07971 (Transamidinase, Glycine amidinotransferase, mitochondrial); L07736 (Cpt1a, Carnitine palmitoyltransferase 1 alpha (liver)); AI237535 (LitaF, LPS-induced TNF-alpha factor); AI175486 (Rps7, Ribosomal protein S7); U32498 (RSEC8, rat homolog of yeast sec8); X53504 (RPL12, Ribosomal protein L12); AF023621 (Sort1, sortilin); AF083269

(P41-Arc, actin-related protein complex 1b); AA891810 (GST, Glutathione S-transferase); M77694 (Fah, fumarylacetoacetate hydrolase); M22357 (MAG, myelin-associated glycoprotein); AI230712 (Pace4, Subtilisin - like endoprotease); AF008439 (NRAMP2, Natural resistance-associated macrophage protein 2); U77829 (Gas-5, growth arrest homolog); U92081 (Gp38, Glycoprotein 38); AA891445 (Skd3, suppressor of K^+ transport defect 3); AI177161 (Nfe2l2, NF-E2-related factor 2); AF031430 (Stx7, Syntaxin 7); L35921 (Ggamma, GTP-binding protein (gamma subunit)); X62322 (Grn, Granulin); AF028784 (GFAP, glial fibrillary acidic protein); and AI234146 (Csrp1, Cysteine rich protein 1).

[108] Using the method of the invention, we have further identified a set of genes and ESTs that changed with age ($p \leq .01$). These include AA891651 (rc_AA891651 EST195454 cDNA); AI070108 (rc_AI070108 UI-R-Y0-lu-a-09-0-UI.s1 cDNA); AI176689 (mitogen-activated protein kinase kinase 6); AI012051 (rc_AI012051 EST206502 cDNA); AI233365 (rc_AI233365 EST230053 cDNA); AA892532 (rc_AA892532 EST196335 cDNA); AA893185 (rc_AA893185 EST196988 cDNA); AA964320 (rc_AA964320 UI-R-C0-gu-e-09-0-UI.s1 cDNA); AA963449 (rc_AA963449 UI-R-E1-gj-e-08-0-UI.s1 cDNA); AA859632 (rc_AA859632 UI-R-E0-bs-h-08-0-UI.s1 cDNA); AI169265 (Atp6s1); AA850781 (rc_AA850781 EST193549 cDNA); AJ222813 (interleukin 18); D38380 (Transferrin); J03481 (dihydropteridine reductase); M24542 (Rieske iron-sulfur protein); L03294 (Lipoprotein lipase); L19998 (sulfotransferase family 1A, phenol-preferring, member 1); U53922 (DnaJ-like protein (RDJ1)); X54793 (liver heat shock protein (hsp60)); X62952 (vimentin); M55534 (Crystallin, alpha polypeptide 2); J03752 (microsomal glutathione S-transferase 1); X64401 (Cytochrome P450, subfamily IIIA, polypeptide 3); X78848 (Gsta1); AF016387 (retinoid X receptor gamma); AF031430 (syntaxin 7); AF051561 (solute carrier family 12, member 2); AF076183 (cytosolic sorting protein PACS-1a (PACS-1)); AF095576 (adaptor protein with pleckstrin homology and src homology 2 domains); AF095741 (MG87); AF097593 (cadherin 2, type 1, N-cadherin (neuronal)); AF104362 (osteoadherin); D10699 (ubiquitin carboxy-terminal hydrolase L1); D28111 (myelin-associated oligodendrocytic basic protein); D37951 (MIBP1 (c-myc intron binding protein 1)); D84477 (RhoA); L13202 (RATHFH2 HNF-3/fork-head homolog-2 (HFH-2)); L26292 (Kruppel-like factor 4 (gut)); L46873 (solute carrier family 15 (oligopeptide transporter), member 1); M13100 (RATLIN3A long interspersed repetitive DNA sequence LINE3 (L1Rn)); M27207 (procollagen, type I, alpha 1); M92433 (Zinc-finger transcription factor NGFI-C (early

response gene)); M94918 (Hemoglobin, beta); M94919 (Hemoglobin, beta); S55427 (Peripheral myelin protein); S68245 (carbonic anhydrase 4); S82649 (Narp=neuronal activity-regulated pentraxin); U10894 (allograft inflammatory factor 1); U26356 (RNSHUNA1 S100A1 gene); U75397 (RNKROX2 Krox-24); U75405 (procollagen, type I, alpha 1); U77829 (RNU77829 gas-5 growth arrest homolog non-translated sequence); U92081 (glycoprotein 38); X06554 (RNMAGSR myelin-associated glycoprotein (S-MAG) C-term); X13167 (Nuclear Factor IA); X14181 (RRRPL18A ribosomal protein L18a); X56325 (Hemoglobin, alpha 1); X60351 (Crystallin, alpha polypeptide 2); E13541 (chondroitin sulfate proteoglycan 5); M22357 (1B236/myelin-associated glycoprotein (MAG)); M24026 (RT1 class Ib gene); M24324 (MHC class I RT1 (RTS) (u haplotype)); J04488 (Prostaglandin D synthase); M15191 (Tachykinin (substance P, neurokinin A, neuropeptide K, neuropeptide gamma)); M74223 (VGF); U17254 (immediate early gene transcription factor NGFI-B); U08259 (Glutamate receptor, ionotropic, N-methyl D-aspartate 2C); U19866 (activity regulated cytoskeletal-associated protein); L40364 (RT1 class Ib gene); U17919 (allograft inflammatory factor 1); U78102 (early growth response 2); U67082 (KRAB-zinc finger protein KZF-1); U77777 (interleukin 18); D78018 (Nuclear Factor IA); U92564 (Olf-1/EBF associated Zn finger protein Roaz); AF008439 (Solute carrier family 11 member 2 (natural resistance-associated macrophage protein 2)); AB003726 (RuvB-like protein 1); M83561 (Glutamate receptor, ionotropic, kainate 1); AI639151 (mixed-tissue library cDNA clone rx02802 3); AI639247 (mixed-tissue library cDNA clone rx03939 3); AI639381 (mixed-tissue library cDNA clone rx01495 3); AI639532 (mixed-tissue library cDNA clone rx01030 3); AA799645 (FXYD domain-containing ion transport regulator 1); AA900516 (Pdi2); AI014169 (Vdup1); AI030089 (Nopp140); AI102299 (Bid3); AA818025 (CD59 antigen); AI170268 (Prostaglandin F receptor); AI171462 (CD24 antigen); AI171966 (ESTs, Highly similar to SPS2 MOUSE SELENIDE,WATER DIKINASE 2 [M.musculus]); AI176456 (ESTs, Weakly similar to ABP2_HUMAN ENDOTHELIAL ACTIN-BINDING PROTEIN [H.sapiens]); AI177161 (NF-E2-related factor 2); AI179576 (Hemoglobin, beta); AI230712 (Subtilisin - like endoprotease); AI230914 (farnesyltransferase beta subunit); AI231213 (kangai 1 (suppression of tumorigenicity 6), prostate); AI237731 (Lipoprotein lipase); M83745 (Protein convertase subtilisin / kexin, type I); M27905 (ribosomal protein L21); M32016 (Lysosomal-associated membrane protein 2); M11071 (RT1 class Ib gene); M15562 (MHC class II RT1.u-D-alpha chain); M15880 (Neuropeptide Y); L08595 (nuclear

receptor subfamily 4, group A, member 2); M18416 (Early growth response 1); L40362 (MHC class I RT1.C-type protein); Z50144 (kynurenine/alpha-aminoadipate aminotransferase); X71127 (complement component 1, q subcomponent, beta polypeptide); U44948 (smooth muscle cell LIM protein (SmLIM)); AA850219 (Annexin A3); X73371 (FCGR2); X57281 (Glycine receptor alpha 2 subunit (glycine receptor, neonatal)); X83231 (pre-alpha-inhibitor); X52477 (Complement component 3); X16554 (phosphoribosyl pyrophosphate synthetase 1); X78605 ((Sprague Dawley) rab4b ras-homologous GTPase); X82445 (nuclear distribution gene C homolog (Aspergillus)); X52619 (ribosomal protein L28); X68283 (ribosomal protein L29); X13044 (CD74 antigen (invariant polpypeptide of major histocompatibility class II antigen-associated)); X54096 (Lecithin-cholesterol acyltransferase); U31866 (Nclone10); U72620 (Lot1); U66470 (rCGR11); M31018 (RT1 class Ib gene); U90887 (arginase type II); M18467 (Glutamate oxaloacetate transaminase 2, mitochondrial (aspartate aminotransferase 2)); M64780 (Agrin); U87627 (putative monocarboxylate transporter (MCT3)); AF019974 (Chromogranin B, parathyroid secretory protein); L03201 (cathepsin S); AB008538 (HB2); D89340 (dipeptidylpeptidase III); M77694 (fumarylacetoacetate hydrolase); M32062 (Fc-gamma receptor); L21192 (brain abundant, membrane attached signal protein 2); M37584 (H2afz); AA858588 (ESTs, Weakly similar to ODP2 RAT DIHYDROLIPOAMIDE ACETYLTRANSFERASE COMPONENT OF PYRUVATE DEHYDROGENASE COMPLEX [R.norvegicus]); AA858617 (rc_AA858617 UI-R-E0-bq-b-06-0-UI.s1 cDNA); AA859562 (rc_AA859562 UI-R-E0-bv-b-03-0-UI.s1 cDNA); AA859626 (rc_AA859626 UI-R-E0-bs-h-02-0-UI.s1 cDNA); AA859690 (rc_AA859690 UI-R-E0-bx-e-11-0-UI.s1 cDNA); AA859777 (rc_AA859777 UI-R-E0-bu-e-10-0-UI.s1 cDNA); AA859975 (LOC64201); AA860030 (UI-R-E0-bz-e-07-0-UI.s2 cDNA); AA866291 (rc_AA866291 UI-R-A0-ac-e-12-0-UI.s3 cDNA); AA866409 (rc_AA866409 UI-R-E0-ch-a-03-0-UI.s1 cDNA); AA866411 (NDN); AA874794 (Bex3); A874887 (rc_AA874887 UI-R-E0-ci-g-10-0-UI.s1 cDNA); AA875004 (rc_AA875004 UI-R-E0-cb-b-07-0-UI.s1 cDNA); AA875037 (rc_AA875037 UI-R-E0-cb-a-03-0-UI.s1 cDNA); AA875047 (TCPZ); AA875059 (rc_AA875059 UI-R-E0-cb-f-04-0-UI.s1 cDNA); AA875129 (rc_AA875129 UI-R-E0-bu-e-01-0-UI.s2 cDNA); H31418 (rc_H31418 EST105434 cDNA); H31665 (rc_H31665 EST105952 cDNA); H32977 (rc_H32977 EST108553 cDNA); H33725 (associated molecule with the SH3 domain of STAM); AA891037 (rc_AA891037 EST194840 cDNA); AA891445 (Skd3); AA891690 (ESTs, Weakly similar to

SERC_HUMAN PHOSPHOSERINE AMINOTRANSFERASE [H.sapiens]); AA891717 (USF1); AA891734 (rc_AA891734 EST195537 cDNA); AA891785 (rc_AA891785 EST195588 cDNA); AA891810 (ESTs, Highly similar to GTK1 RAT GLUTATHIONE S-TRANSFERASE, MITOCHONDRIAL [R.norvegicus]); AA891965 (rc_AA891965 EST195768 cDNA); AA892333 (Tuba1); AA892353 (ESTs, Moderately similar to JC5823 NADH dehydrogenase [H.sapiens]); AA892511 (rc_AA892511 EST196314 cDNA); AA892986 (rc_AA892986 EST196789 cDNA); AA893032 (ESTs, Moderately similar to CALX RAT CALNEXIN PRECURSOR [R.norvegicus]); AA893082 (rc_AA893082 EST196885 cDNA); AA893493 (RPL26); AA893607 (rc_AA893607 EST197410 cDNA); AA893708 (KIAA0560); AA893743 (rc_AA893743 EST197546 cDNA); AA894104 (rc_AA894104 EST197907 cDNA); AA799449 (EST, Weakly similar to UBP4 MOUSE UBIQUITIN CARBOXYL-TERMINAL HYDROLASE 4 [M.musculus]); AA799779 (acyl-CoA:dihydroxyacetonephosphate acyltransferase); AA799803 (ESTs, Weakly similar to K1CU RAT KERATIN, TYPE I CYTOSKELETAL 21 [R.norvegicus]); AA799854 (rc_AA799854 EST189351 cDNA); AA799996 (rc_AA799996 EST189493 cDNA); AA800693 (rc_AA800693 EST190190 cDNA); AA800708 (ESTs, Weakly similar to S28312 hypothetical protein F02A9.4 -Caenorhabditis elegans [C.elegans]); AA800794 (HT2A); and AA800948 (Tuba4).

[109] We have also identified age-related ESTs, including AA963449 (UI-R-E1-gj-e-08-0-UI.s1 cDNA); AA892532 (EST196335 cDNA); AA859626 (UI-R-E0-bs-h-02-0-UI.s1 cDNA); AA893743 (EST197546 cDNA); AI233365 (EST230053 cDNA); H31665 (EST105952 cDNA); AA892353 (ESTs, Moderately similar to JC5823 NADH dehydrogenase); AI639247 (mixed-tissue library cDNA clone rx03939 3); AA858617 (UI-R-E0-bq-b-06-0-UI.s1 cDNA); AI639429 (mixed-tissue library cDNA clone rx00973 3); AA858620 (UI-R-E0-bq-b-09-0-UI.s1 cDNA); AA866291 (UI-R-A0-ac-e-12-0-UI.s3 cDNA); AA894104 (EST197907 cDNA); AA799996 (EST189493 cDNA); AA892805 (EST196608 cDNA); AI639019 (mixed-tissue library cDNA clone rx01107 3); AA799538 (EST189035 cDNA); AI070108 (UI-R-Y0-lu-a-09-0-UI.s1 cDNA); AA866409 (UI-R-E0-ch-a-03-0-UI.s1 cDNA); AA859632 (UI-R-E0-bs-h-08-0-UI.s1 cDNA); AA891651 (EST195454 cDNA); AA893032 (ESTs, Moderately similar to CALX calnexin precursor); AA891965 (EST195768 cDNA); AA800708 (ESTs, Weakly similar to S28312 hypothetical protein F02A9.4); AA964320 (UI-R-C0-gu-e-09-0-UI.s1 cDNA); AA893173 (EST196976

cDNA); H32977 (EST108553 cDNA); AA874887 (UI-R-E0-ci-g-10-0-UI.s1 cDNA); AA850781 (EST193549 cDNA); AI176456 (ESTs, Weakly similar to endothelial actin-binding protein); H31418 (EST105434 cDNA); AA858588 (ESTs, Weakly similar to ODP2 dihydrolipoamide acetyl transferase); AA891785 (EST195588 cDNA); AA799803 (ESTs, Weakly similar to K1CU cytoskeletal keratin (type 1)); AA799449 (EST, Weakly similar to UBP4 ubiquitin carboxyl-terminal hydrolase 4); AA859777 (UI-R-E0-bu-e-10-0-UI.s1 cDNA); AI639532 (mixed-tissue library cDNA clone rx01030 3); AA875059 (UI-R-E0-cb-f-04-0-UI.s1 cDNA); AI012051 (EST206502 cDNA); AA800549 (EST190046 cDNA); AA799854 (EST189351 cDNA); and AA892520 (EST196323 cDNA).

[110] Those of skill in the genomics art will understand that the identified genes and ESTs have utility as biomarkers of brain aging. Those of skill in the genomics art will understand that the mammalian homologues (including rat, mouse and human homologues) the identified genes and ESTs are also as biomarkers of brain aging. The easiest method for identifying mammalian homologues of the identified genes and ESTs is by identifying the homologues in the GenBank database, preferably, or in the SwissProtein and the Genome Ontology databases. Additional guidance as to homology can be obtained by using commercially available computer programs, such as DNA Strider and Wisconsin GCG, and following the instructions for the determination of the degree of homolgy between selected polynucleotides.

[111] The foregoing description has been presented only for the purposes of illustration and is not intended to limit the invention to the precise form disclosed, but by the claims appended hereto.

CLAIMS

What is claimed is:

1. A method for identifying a biomarker for brain aging, wherein the biomarker is a polynucleotide or a polypeptide encoded by said polynucleotide, comprising the steps of:

(a) obtaining a set of polynucleotides obtained from a set of brain samples, wherein the members of the set of brain samples were obtained from members of a set of mammals, wherein the set of mammals contains more than two members and wherein the set of mammals comprises young, mid-aged and aged members;

(b) identifying the identity and amount of the members of the set of polynucleotides present in the brain samples;

(c) deleting from the set of polynucleotides;

(1) quality control oligonucleotides;

(2) polynucleotides in which the difference between the young and the aged members did not comprise at least 75% of the maximum normalized difference among the members; and

(d) testing by a conventional statistical test for a significant effect of aging across the young, mid-aged and aged members;

wherein the polynucleotides, and polypeptide encoded by said polynucleotides, that are both significantly altered in an age-dependent fashion across age are identified as biomarkers for brain aging.

2. The identification method of claim 1, further comprising the step of:

(e) correlating the identity and amount of the members of the set of polynucleotides present in the brain samples with cognitive performance in a behavioral test, using a conventional statistical correlation test;

wherein the polynucleotides, and polypeptide encoded by said polynucleotides, that are both significantly altered in an age-dependent fashion as well as significantly correlated with cognitive performance across age are identified as biomarkers for brain aging.

3. The identification method of claim 1, wherein the biomarker for brain aging is a biomarker for an age-related neurodegenerative condition.

4. The identification method of claim 3, wherein the age-related neurodegenerative condition is Alzheimer's disease or Parkinson's disease.

5. The identification method of claim 1, wherein the brain sample is a hippocampal sample.

6. The identification method of claim 1, wherein the mammal is selected from the group consisting of rat, mouse and human.

7. The identification method of claim 1, wherein the biomarker for brain aging is an expressed sequence tag (EST).

8. The identification method of claim 1, further comprising, in the deletion step (c), the step of:

(3) deleting, from the set of polynucleotides, polynucleotides for expressed sequence tags (ESTs) which have not been associated with known genes.

9. The identification method of claim 1, further comprising, in the deletion step (c), the step of:

(3) deleting, from the set of polynucleotides, polynucleotides that are not expressed sequence tags (ESTs).

10. The identification method of claim 1, wherein the conventional statistical test in step (d) is ANOVA or student's t test.

11. The identification method of claim 1, wherein the testing in step (d) is testing by 1-way ANOVA for a significant effect of aging $p < 0.05$.

12. The identification method of claim 1, wherein the behavioral tests of step (e) specifically test for cognitive deficits related to the region of the brain from which the brain sample has been obtained in step (a).

13. The identification method of claim 1, wherein the identification of the identity and amount of the members of the set of polynucleotides present in the brain samples in step (b) is by microarray analysis.

14. The identification method of claim 1, wherein the significant effect in step (d) is $p < 0.025$.

15. The identification method of claim 1, wherein the significant effect in step (d) is $p < 0.01$.

16. The identification method of claim 1, wherein the significant effect in step (d) is $p < 0.001$.

17. The identification method of claim 1, wherein the behavioral test in step (e) is selected from the group consisting of the Morris spatial water maze (SWM) and the object memory task (OMT).

18. The identification method of claim 1, wherein the behavioral tests in step (e) are selected from the group consisting of tests for Alzheimer's disease and tests for Parkinson's disease.

19. The identification method of claim 1, wherein the correlation of the identity and amount of the members of the set of polynucleotides present in the brain samples with cognitive performance in behavioral tests is a Pearson or Spearman correlation of expression with behavior.

20. The identification method of claim 19, wherein the correlation is $p < 0.025$.

21. The identification method of claim 19, wherein the correlation is $p < 0.01$.

22. The identification method of claim 19, wherein the correlation is $p < 0.001$.

23. A set of biomarkers for brain aging, comprising mammalian polynucleotides and polypeptides encoded by said polynucleotides:
    (a) wherein the set of biomarkers comprises at least two members;
    (b) wherein the brain expression patterns of the members of the set are significantly altered with aging as determined by a conventional statistical test, with $p < 0.05$;
    (c) wherein the brain expression patterns of the members of the set are correlated across age groups with cognitive performance in behavioral tests, using a conventional statistical correlation test with a correlation of $p < 0.05$ between brain expression and cognitive performance; and
    (d) wherein the cognitive performance in behavioral tests significantly altered with aging as determined by a conventional statistical test.

24. The set of biomarkers of claim 23, wherein the biomarkers further correlate with a behavioral measure of functional impairment.

25. The set of biomarkers of claim 23, wherein the behavioral measure of functional impairment is a test for an age-related neurodegenerative condition.

26. The set of biomarkers of claim 25, wherein the age-related neurodegenerative condition is Alzheimer's disease or Parkinson's disease.

27. The set of biomarkers of claim 23, wherein the mammal is selected from the group consisting of rat, mouse and human.

28. The set of biomarkers of claim 23, wherein the conventional statistical method in step (b) is ANOVA or student's t-test.

29. The set of biomarkers of claim 23, wherein the correlation in step (c) is tested by a correlation test selected from the group consisting of Pearson's correlation test and Spearman's correlation test.

30. The set of biomarkers of claim 23, wherein the age groups in step (c) comprises young, mid-aged and aged.

31. The set of biomarkers of claim 23, wherein the conventional statistical test in step (d) is ANOVA or student's t-test.

32. The set of biomarkers of claim 23, wherein at least one member of the set of biomarkers is a polynucleotide, or a polypeptide encoded by said polynucleotide, selected from the group consisting of L03294 (Lpl, lipoprotein lipase); M18416 (Egr1, Early growth response 1 (Krox-24)); S68245 (Ca4, carbonic anhydrase 4); M64780 (Agrn, Agrin); M27207 (Col1a1, Procollagen- type I (alpha 1)); X16554 (Prps1, Phosphoribosyl pyrophosphate synthetase 1); M92433 (NGFI-C, Zinc-finger transcription factor (early response gene)); AA859975 (LOC64201, 2-oxoglutarate carrier); L08595 (Nuclear receptor subfamily 4, group A, member 2); M24542 (RISP, Rieske iron-sulfur protein); AI030089 (Nopp130, nucleolar phosphoprotein p130); AF104362 (Omd, Osteomodulin (osteoadherin)); L46873 (Slc15a1, Oligopeptide transporter); AI176689 (MAPKK 6, mitogen-activated protein kinase kinase 6); U66470 (rCGR11, Cell growth regulator); AF016387 (RXRG, retinoid X-receptor gamma); M18467 (Got2, glutamate oxaloacetate transaminase 2); X54793 (Hsp60, heat shock protein 60); X64401 (Cyp3a3, Cytochrome P450- subfamily IIIA (polypeptide 3)); M37584 (H2afz, H2A histone family (member Z)); L21192 (GAP-43, membrane attached signal protein 2 (brain)); AA875047 (TCPZ, T-complex protein 1 (zeta subunit)); U90610 (Cxcr4, CXC chemokine receptor); AF003904 (CRH-binding protein); U83880 (GPDH-M, glycerol-3-phosphate dehydrogenase, mitochondrial); X89703 (TPCR19, Testis Polymerase Chain Reaction product 19);

D63886 (MMP16, matrix metalloproteinase 16); J05499 (GLS, glutaminase (mitochondrial)); D21799 (Psmb2, Proteasome subunit (beta type 2)); AA800794 (HT2A, zinc-finger protein); U90887 (Arg2, arginase type II); S82649 (Narp, neuronal activity-regulated pentraxin); M74223 (VGF, neurosecretory protein); AA874794 (Bex3, brain expressed X-linked 3); M15191 (Tac1, Tachykinin); AA892506 (coronin, actin binding protein 1A); L04485 (MAPPK1, mitogen-activated protein kinase kinase 1); AA799641 (S164, Contains a PWI domain associated with RNA splicing); AA817892 (Gnb2, Guanine nucleotide binding protein (beta 2 subunit)); AA893939 (DSS1, deleted in split hand/ split foot protein 1); AF000901 (P58/P45, Nucleoporin p58); AF087037 (Btg3, B-cell translocation gene 3); AB000280 (PHT1, peptide/histidine transporter); M87854 (Beta-ARK-1, beta adrenergic receptor kinase 1); U06099 (Prdx2, Peroxiredoxin 2); AF058795 (Gb2, GABA-B receptor); AA800517 (VAP1, vesicle associated protein); U63740 (Fez1, Protein kinase C-binding protein Zeta1); U53922 (Hsj2, DnaJ-like protein (RDJ1)); U78102 (Egr2, Early growth response 2); U44948 (SmLIM, smooth muscle cell LIM protein); U87627 (MCT3, putative monocarboxylate transporter); AB020504 (PMF31, highly homologus to mouse F-box-WD40 repeat protein 6); M21354 (Col3a1, collagen type III alpha-1); AA893664 (Temo, sertoli cell marker (KIAA0077 protein fragment)); AB010437 (CDH8, Cadherin-8); M22756 (Ndufv2, mitochondrial NADH dehydrogenase (24 kDa)); AA799389 (Rab3B, ras-related protein); AI172476 (Tieg-1, TGF-beta-inducible early growth response protein 1); AF091563 (Olfactory receptor); M64376 (Olfactory protein); J04488 (Ptgds, Prostaglandin D synthase); X71127 (c1qb, complement component 1- q (beta polypeptide)); J03752 (Microsomal GST-1, glutathione S-transferase); J03481 (Qdpr, Dihydropteridine reductase); L40362 (MHC class I RT1.C-type protein); M94918 (Hbb, beta hemoglobin); M55534 (Cryab, alpha crystallin polypeptide 2); U17919 (Aif1, allograft inflammatory factor 1); M15562 (MHC class II RT1.u-D-alpha chain); AA799645 (Phospholemman, FXYD domain-containing ion transport regulator 1); X13044 (Cd74, CD74 antigen); M24324 (RTS, MHC class I RT1 (RTS) (u haplotype)); U31866 (Nclone10); M32062 (Fcgr3, Fc IgG receptor III (low affinity)); AF095741 (Mg87); L03201 (Ctss, cathepsin S); M27905 (Rpl21, Ribosomal protein L21); D38380 (Tf, Transferrin); AA893493 (RPL26, Ribosomal

protein L26); AJ222813 (Il18, interleukin 18); E13541 (Cspg5, chondroitin sulfate proteoglycan 5); X54096 (Lcat, Lecithin-cholesterol acyltransferase); L40364 (RT1Aw2, RT1 class Ib); D28111 (MOBP, myelin-associated oligodendrocytic basic protein); M32016 (Lamp2, lysosomal-associated membrane protein 2); X13167 (NF1-A, nuclear factor 1 A); U26356 (S100A1, S100 protein (alpha chain)); AI231213 (Kangai 1, suppression of tumorigenicity 6); AI170268 (Ptgfr, Prostaglandin F receptor); X62952 (Vim, vimentin); AI014169 (Vdup1, vitamin D-upregulated); AA850219 (Anx3, Annexin A3); D84477 (Rhoa, ras-related homolog A2); X52477 (C3, Complement component 3); X52619 (Rpl28, Ribosomal protein L28); X06554 (S-MAG, myelin-associated glycoprotein C-term); Z50144 (Kat2, kynurenine aminotransferase II); X14181 (RPL18A, Ribosomal protein L18a); AA892333 (Tuba1, alpha-tubulin); U67082 (KZF-1, Kruppel associated box (KRAB) zinc finger 1); U11760 (Vcp, valosin-containing protein); AF048828 (VDAC1, voltage-dependent anion channel 1); M31076 (TNF-alpha, Transforming growth factor (alpha)); S83279 (HSDIV, 17-beta-hydroxysteroid dehydrogenase type IV); AI102103 (Pik4cb, Phosphatidylinositol 4-kinase); X56325 (Hba1, alpha 1 hemoglobin); X73371 (FCGR2, Low affinity immunoglobulin gamma Fc receptor II); X78848 (Gsta1, Glutathione-S-transferase (alpha type)); U92564 (Roaz, Olf-1/EBF associated Zn finger protein); AI171462 (Cd24, CD24 antigen); X83231 (PAIHC3, Pre-alpha-inhibitor, heavy chain 3); AF097593 (Ca4, cadherin 2- type 1 (neuronal)); X68283 (Rpl29, Ribosomal protein L29); S55427 (Pmp, peripheral myelin protein); AA818025 (Cd59, CD59 antigen); E01534 (Rps15, Ribosomal protein S15); U37138 (Sts, Steroid sulfatase); X55572 (Apod, Apolipoprotein D); AI028975 (AP-1, adaptor protein complex (beta 1)); L16995 (ADD1, adipocyte determination/ differentiation-dependent factor 1); U07971 (Transamidinase, Glycine amidinotransferase, mitochondrial); L07736 (Cpt1a, Carnitine palmitoyltransferase 1 alpha (liver)); AI237535 (LitaF, LPS-induced TNF-alpha factor); AI175486 (Rps7, Ribosomal protein S7); U32498 (RSEC8, rat homolog of yeast sec8); X53504 (RPL12, Ribosomal protein L12); AF023621 (Sort1, sortilin); AF083269 (P41-Arc, actin-related protein complex 1b); AA891810 (GST, Glutathione S-transferase); M77694 (Fah, fumarylacetoacetate hydrolase); M22357 (MAG, myelin-associated glycoprotein); AI230712 (Pace4, Subtilisin - like endoprotease); AF008439

(NRAMP2, Natural resistance-associated macrophage protein 2); U77829 (Gas-5, growth arrest homolog); U92081 (Gp38, Glycoprotein 38); AA891445 (Skd3, suppressor of K^+ transport defect 3); AI177161 (Nfe2l2, NF-E2-related factor 2); AF031430 (Stx7, Syntaxin 7); L35921 (Ggamma, GTP-binding protein (gamma subunit)); X62322 (Grn, Granulin); AF028784 (GFAP, glial fibrillary acidic protein); AI234146 (Csrp1, Cysteine rich protein 1) and mammalian homologues thereof.

33. The set of biomarkers of claim 23, wherein at least one member of the set of biomarkers is an expressed sequence tag (EST).

34. The set of biomarkers of claim 23, for use in the measurement of age-dependent cognitive decline.

35. The set of biomarkers of claim 34, wherein the age-dependent cognitive decline is an age-related neurodegenerative condition.

36. The set of biomarkers of claim 35, wherein the age-related neurodegenerative condition is Alzheimer's disease or Parkinson's disease.

37. The set of biomarkers of claim 23, for use in the measurement of degree of the safety or effectiveness of compounds or procedures directed against age-related cognitive decline.

38. A set of biomarkers for brain aging, comprising mammalian polynucleotides and polypeptides encoded by said polypeptides:
    (a) wherein the set of biomarkers comprises at least two members;
    (b) wherein the brain expression patterns of the members of the set are significantly altered with aging as measured by a conventional statistical method at a significance level of $p < 0.01$.

39. The set of biomarkers of claim 38, wherein the mammal is selected from the group consisting of rat, mouse and human.

40. The set of biomarkers of claim 38, wherein the conventional statistical method is ANOVA or student's t-test.

41. The set of biomarkers of claim 38, wherein at least one member of the set of biomarkers is a polynucleotide, or a polypeptide encoded by said polynucleotide, selected from the group consisting of AA891651 (rc_AA891651 EST195454 cDNA); AI070108 (rc_AI070108 UI-R-Y0-lu-a-09-0-UI.s1 cDNA); AI176689 (mitogen-activated protein kinase kinase 6); AI012051 (rc_AI012051 EST206502 cDNA); AI233365 (rc_AI233365 EST230053 cDNA); AA892532 (rc_AA892532 EST196335 cDNA); AA893185 (rc_AA893185 EST196988 cDNA); AA964320 (rc_AA964320 UI-R-C0-gu-e-09-0-UI.s1 cDNA); AA963449 (rc_AA963449 UI-R-E1-gj-e-08-0-UI.s1 cDNA); AA859632 (rc_AA859632 UI-R-E0-bs-h-08-0-UI.s1 cDNA); AI169265 (Atp6s1); AA850781 (rc_AA850781 EST193549 cDNA); AJ222813 (interleukin 18); D38380 (Transferrin); J03481 (dihydropteridine reductase); M24542 (Rieske iron-sulfur protein); L03294 (Lipoprotein lipase); L19998 (sulfotransferase family 1A, phenol-preferring, member 1); U53922 (DnaJ-like protein (RDJ1)); X54793 (liver heat shock protein (hsp60)); X62952 (vimentin); M55534 (Crystallin, alpha polypeptide 2); J03752 (microsomal glutathione S-transferase 1); X64401 (Cytochrome P450, subfamily IIIA, polypeptide 3); X78848 (Gsta1); AF016387 (retinoid X receptor gamma); AF031430 (syntaxin 7); AF051561 (solute carrier family 12, member 2); AF076183 (cytosolic sorting protein PACS-1a (PACS-1)); AF095576 (adaptor protein with pleckstrin homology and src homology 2 domains); AF095741 (MG87); AF097593 (cadherin 2, type 1, N-cadherin (neuronal)); AF104362 (osteoadherin); D10699 (ubiquitin carboxy-terminal hydrolase L1); D28111 (myelin-associated oligodendrocytic basic protein); D37951 (MIBP1 (c-myc intron binding protein 1)); D84477 (RhoA); L13202 (RATHFH2 HNF-3/fork-head homolog-2 (HFH-2)); L26292 (Kruppel-like factor 4 (gut)); L46873 (solute carrier family 15 (oligopeptide transporter), member 1); M13100 (RATLIN3A long interspersed repetitive DNA sequence LINE3 (L1Rn)); M27207 (procollagen, type I, alpha 1); M92433 (Zinc-finger transcription factor NGFI-C (early response gene)); M94918 (Hemoglobin, beta); M94919 (Hemoglobin, beta); S55427 (Peripheral

myelin protein); S68245 (carbonic anhydrase 4); S82649 (Narp=neuronal activity-regulated pentraxin); U10894 (allograft inflammatory factor 1); U26356 (RNSHUNA1 S100A1 gene); U75397 (RNKROX2 Krox-24); U75405 (procollagen, type I, alpha 1); U77829 (RNU77829 gas-5 growth arrest homolog non-translated sequence); U92081 (glycoprotein 38); X06554 (RNMAGSR myelin-associated glycoprotein (S-MAG) C-term); X13167 (Nuclear Factor IA); X14181 (RRRPL18A ribosomal protein L18a); X56325 (Hemoglobin, alpha 1); X60351 (Crystallin, alpha polypeptide 2); E13541 (chondroitin sulfate proteoglycan 5); M22357 (1B236/myelin-associated glycoprotein (MAG)); M24026 (RT1 class Ib gene); M24324 (MHC class I RT1 (RTS) (u haplotype)); J04488 (Prostaglandin D synthase); M15191 (Tachykinin (substance P, neurokinin A, neuropeptide K, neuropeptide gamma)); M74223 (VGF); U17254 (immediate early gene transcription factor NGFI-B); U08259 (Glutamate receptor, ionotropic, N-methyl D-aspartate 2C); U19866 (activity regulated cytoskeletal-associated protein); L40364 (RT1 class Ib gene); U17919 (allograft inflammatory factor 1); U78102 (early growth response 2); U67082 (KRAB-zinc finger protein KZF-1); U77777 (interleukin 18); D78018 (Nuclear Factor IA); U92564 (Olf-1/EBF associated Zn finger protein Roaz); AF008439 (Solute carrier family 11 member 2 (natural resistance-associated macrophage protein 2)); AB003726 (RuvB-like protein 1); M83561 (Glutamate receptor, ionotropic, kainate 1); AI639151 (mixed-tissue library cDNA clone rx02802 3); AI639247 (mixed-tissue library cDNA clone rx03939 3); AI639381 (mixed-tissue library cDNA clone rx01495 3); AI639532 (mixed-tissue library cDNA clone rx01030 3); AA799645 (FXYD domain-containing ion transport regulator 1); AA900516 (Pdi2); AI014169 (Vdup1); AI030089 (Nopp140); AI102299 (Bid3); AA818025 (CD59 antigen); AI170268 (Prostaglandin F receptor); AI171462 (CD24 antigen); AI171966 (ESTs, Highly similar to SPS2 MOUSE SELENIDE,WATER DIKINASE 2 [M.musculus]); AI176456 (ESTs, Weakly similar to ABP2_HUMAN ENDOTHELIAL ACTIN-BINDING PROTEIN [H.sapiens]); AI177161 (NF-E2-related factor 2); AI179576 (Hemoglobin, beta); AI230712 (Subtilisin - like endoprotease); AI230914 (farnesyltransferase beta subunit); AI231213 (kangai 1 (suppression of tumorigenicity 6), prostate); AI237731 (Lipoprotein lipase); M83745 (Protein convertase subtilisin / kexin, type I); M27905 (ribosomal protein L21);

M32016 (Lysosomal-associated membrane protein 2); M11071 (RT1 class Ib gene); M15562 (MHC class II RT1.u-D-alpha chain); M15880 (Neuropeptide Y); L08595 (nuclear receptor subfamily 4, group A, member 2); M18416 (Early growth response 1); L40362 (MHC class I RT1.C-type protein); Z50144 (kynurenine/alpha-aminoadipate aminotransferase); X71127 (complement component 1, q subcomponent, beta polypeptide); U44948 (smooth muscle cell LIM protein (SmLIM)); AA850219 (Annexin A3); X73371 (FCGR2); X57281 (Glycine receptor alpha 2 subunit (glycine receptor, neonatal)); X83231 (pre-alpha-inhibitor); X52477 (Complement component 3); X16554 (phosphoribosyl pyrophosphate synthetase 1); X78605 ((Sprague Dawley) rab4b ras-homologous GTPase); X82445 (nuclear distribution gene C homolog (Aspergillus)); X52619 (ribosomal protein L28); X68283 (ribosomal protein L29); X13044 (CD74 antigen (invariant polpypeptide of major histocompatibility class II antigen-associated)); X54096 (Lecithin-cholesterol acyltransferase); U31866 (Nclone10); U72620 (Lot1); U66470 (rCGR11); M31018 (RT1 class Ib gene); U90887 (arginase type II); M18467 (Glutamate oxaloacetate transaminase 2, mitochondrial (aspartate aminotransferase 2)); M64780 (Agrin); U87627 (putative monocarboxylate transporter (MCT3)); AF019974 (Chromogranin B, parathyroid secretory protein); L03201 (cathepsin S); AB008538 (HB2); D89340 (dipeptidylpeptidase III); M77694 (fumarylacetoacetate hydrolase); M32062 (Fc-gamma receptor); L21192 (brain abundant, membrane attached signal protein 2); M37584 (H2afz); AA858588 (ESTs, Weakly similar to ODP2 RAT DIHYDROLIPOAMIDE ACETYLTRANSFERASE COMPONENT OF PYRUVATE DEHYDROGENASE COMPLEX [R.norvegicus]); AA858617 (rc_AA858617 UI-R-E0-bq-b-06-0-UI.s1 cDNA); AA859562 (rc_AA859562 UI-R-E0-bv-b-03-0-UI.s1 cDNA); AA859626 (rc_AA859626 UI-R-E0-bs-h-02-0-UI.s1 cDNA); AA859690 (rc_AA859690 UI-R-E0-bx-e-11-0-UI.s1 cDNA); AA859777 (rc_AA859777 UI-R-E0-bu-e-10-0-UI.s1 cDNA); AA859975 (LOC64201); AA860030 (UI-R-E0-bz-e-07-0-UI.s2 cDNA); AA866291 (rc_AA866291 UI-R-A0-ac-e-12-0-UI.s3 cDNA); AA866409 (rc_AA866409 UI-R-E0-ch-a-03-0-UI.s1 cDNA); AA866411 (NDN); AA874794 (Bex3); A874887 (rc_AA874887 UI-R-E0-ci-g-10-0-UI.s1 cDNA); AA875004 (rc_AA875004 UI-R-E0-cb-b-07-0-UI.s1 cDNA); AA875037 (rc_AA875037 UI-R-E0-cb-a-03-0-UI.s1 cDNA); AA875047

(TCPZ); AA875059 (rc_AA875059 UI-R-E0-cb-f-04-0-UI.s1 cDNA); AA875129 (rc_AA875129 UI-R-E0-bu-e-01-0-UI.s2 cDNA); H31418 (rc_H31418 EST105434 cDNA); H31665 (rc_H31665 EST105952 cDNA); H32977 (rc_H32977 EST108553 cDNA); H33725 (associated molecule with the SH3 domain of STAM); AA891037 (rc_AA891037 EST194840 cDNA); AA891445 (Skd3); AA891690 (ESTs, Weakly similar to SERC_HUMAN PHOSPHOSERINE AMINOTRANSFERASE [H.sapiens]); AA891717 (USF1); AA891734 (rc_AA891734 EST195537 cDNA); AA891785 (rc_AA891785 EST195588 cDNA); AA891810 (ESTs, Highly similar to GTK1 RAT GLUTATHIONE S-TRANSFERASE, MITOCHONDRIAL [R.norvegicus]); AA891965 (rc_AA891965 EST195768 cDNA); AA892333 (Tuba1); AA892353 (ESTs, Moderately similar to JC5823 NADH dehydrogenase [H.sapiens]); AA892511 (rc_AA892511 EST196314 cDNA); AA892986 (rc_AA892986 EST196789 cDNA); AA893032 (ESTs, Moderately similar to CALX RAT CALNEXIN PRECURSOR [R.norvegicus]); AA893082 (rc_AA893082 EST196885 cDNA); AA893493 (RPL26); AA893607 (rc_AA893607 EST197410 cDNA); AA893708 (KIAA0560); AA893743 (rc_AA893743 EST197546 cDNA); AA894104 (rc_AA894104 EST197907 cDNA); AA799449 (EST, Weakly similar to UBP4 MOUSE UBIQUITIN CARBOXYL-TERMINAL HYDROLASE 4 [M.musculus]); AA799779 (acyl-CoA:dihydroxyacetonephosphate acyltransferase); AA799803 (ESTs, Weakly similar to K1CU RAT KERATIN, TYPE I CYTOSKELETAL 21 [R.norvegicus]); AA799854 (rc_AA799854 EST189351 cDNA); AA799996 (rc_AA799996 EST189493 cDNA); AA800693 (rc_AA800693 EST190190 cDNA); AA800708 (ESTs, Weakly similar to S28312 hypothetical protein F02A9.4 - Caenorhabditis elegans [C.elegans]); AA800794 (HT2A); AA800948 (Tuba4); and mammalian homologues thereof.

42. The set of biomarkers of claim 38, wherein at least one member of the set of biomarkers is an expressed sequence tag (EST).

43. The set of biomarkers of claim 38, for use in the measurement of age-dependent cognitive decline.

44. The set of biomarkers of claim 43, wherein the age-dependent cognitive decline is an age-dependent neurodegenerative condition.

45. The set of biomarkers of claim 44, wherein the age-dependent neurodegenerative condition is Alzheimer's disease or Parkinson's disease.

46. The set of biomarkers of claim 38, for use in the measurement of the degree of the safety or effectiveness of compounds or procedures directed against age-related cognitive decline.

47. The use of an expressed sequence tag (EST) in an assay for aging in a mammal, wherein the EST is selected from the group consisting of AA963449 (UI-R-E1-gj-e-08-0-UI.s1 cDNA); AA892532 (EST196335 cDNA); AA859626 (UI-R-E0-bs-h-02-0-UI.s1 cDNA); AA893743 (EST197546 cDNA); AI233365 (EST230053 cDNA); H31665 (EST105952 cDNA); AA892353 (ESTs, Moderately similar to JC5823 NADH dehydrogenase); AI639247 (mixed-tissue library cDNA clone rx03939 3); AA858617 (UI-R-E0-bq-b-06-0-UI.s1 cDNA); AI639429 (mixed-tissue library cDNA clone rx00973 3); AA858620 (UI-R-E0-bq-b-09-0-UI.s1 cDNA); AA866291 (UI-R-A0-ac-e-12-0-UI.s3 cDNA); AA894104 (EST197907 cDNA); AA799996 (EST189493 cDNA); AA892805 (EST196608 cDNA); AI639019 (mixed-tissue library cDNA clone rx01107 3); AA799538 (EST189035 cDNA); AI070108 (UI-R-Y0-lu-a-09-0-UI.s1 cDNA); AA866409 (UI-R-E0-ch-a-03-0-UI.s1 cDNA); AA859632 (UI-R-E0-bs-h-08-0-UI.s1 cDNA); AA891651 (EST195454 cDNA); AA893032 (ESTs, Moderately similar to CALX calnexin precursor); AA891965 (EST195768 cDNA); AA800708 (ESTs, Weakly similar to S28312 hypothetical protein F02A9.4); AA964320 (UI-R-C0-gu-e-09-0-UI.s1 cDNA); AA893173 (EST196976 cDNA); H32977 (EST108553 cDNA); AA874887 (UI-R-E0-ci-g-10-0-UI.s1 cDNA); AA850781 (EST193549 cDNA); AI176456 (ESTs, Weakly similar to endothelial actin-binding protein); H31418 (EST105434 cDNA); AA858588 (ESTs, Weakly similar to ODP2 dihydrolipoamide acetyl transferase); AA891785 (EST195588 cDNA); AA799803 (ESTs, Weakly similar to K1CU cytoskeletal keratin (type 1)); AA799449 (EST, Weakly similar to UBP4 ubiquitin carboxyl-terminal

hydrolase 4); AA859777 (UI-R-E0-bu-e-10-0-UI.s1 cDNA); AI639532 (mixed-tissue library cDNA clone rx01030 3); AA875059 (UI-R-E0-cb-f-04-0-UI.s1 cDNA); AI012051 (EST206502 cDNA); AA800549 (EST190046 cDNA); AA799854 (EST189351 cDNA); AA892520 (EST196323 cDNA) and mammalian homologues thereof.

48. The use of claim 47, wherein the assay for aging is a measurement of age-dependent cognitive decline.

49. The use of claim 48, wherein the age-dependent cognitive decline is an age-dependent neurodegenerative condition.

50. The use of claim 49, wherein the age-dependent neurodegenerative condition is Alzheimer's disease or Parkinson's disease.

51. The use of claim 47, wherein the assay for aging is a measurement of the degree of the safety or effectiveness of compounds or procedures directed against age-related cognitive decline.

52. A set of biomarkers for brain aging, comprising mammalian polynucleotides and polypeptides encoded by said polynucleotides:
    (a) wherein the set of biomarkers comprises at least two members; and
    (b) wherein the brain expression patterns of the members of the set are significantly altered with aging as determined by a conventional statistical method, with $p < 0.05$.

53. The set of biomarkers of claim 52, wherein the set contains at least one member selected from the group consisting of AA685974 (EST108806 cDNA); AA799396 (EST188893 cDNA); AA799479 (ESTs, Highly similar to NADH-ubiquinone oxidoreduct.); AA799481 (EST188978 cDNA); AA799529 (EST189026 cDNA); AA799599 (EST189096 cDNA); AA799636 (EST189133 cDNA); AA799680 (EST189177 cDNA); AA799724 (ESTs, Highly similar to DNA-directed RNA

polymerasel); AA799773 (EST189270 cDNA); AA799779 (acyl-CoA:dihydroxyacetonephosphate acyltransferase); AA799858 (EST189355 cDNA); AA800026 (EST189523 cDNA); AA800318 (EST189815 cDNA); AA800622 (EST190119 cDNA); AA800693 (EST190190 cDNA); AA800948 (Tuba4); AA801286 (Inositol (myo)-1(or 4)-monophosphatase 1); AA817887 (profilin); AA818025 (CD59 antigen); AA818240 (Nuclear pore complex protein); AA818487 (cyclophilin B); AA819500 (ESTs, Highly similar to AC12_HUMAN 37 kD subunit); AA819708 (Cox7a3); AA848831 (lysophosphatidic acid G-protein-coupled receptor, 2); AA852046 (EST194815 cDNA); AA859545 (ESTs, Weakly similar to hypothetical protein C09H6.3); AA859562 (UI-R-E0-bv-b-03-0-UI.s1 cDNA); AA859643 (UI-R-E0-bs-a-08-0-UI.s1 cDNA); AA859645 (attractin); AA859690 (UI-R-E0-bx-e-11-0-UI.s1 cDNA); AA859848 (UI-R-E0-cc-h-10-0-UI.s1 cDNA); AA859954 (Vacuole Membrane Protein 1); AA859980 (T-complex 1); AA860030 (UI-R-E0-bz-e-07-0-UI.s2 cDNA); AA866257 (ESTs); AA866299 (UI-R-A0-ac-f-12-0-UI.s3 cDNA); AA866299 (UI-R-A0-ac-f-12-0-UI.s3 cDNA); AA866432 (UI-R-E0-ch-e-06-0-UI.s1 cDNA); AA866477 (UI-R-E0-br-h-03-0-UI.s1 cDNA); AA874830 (UI-R-E0-cg-f-04-0-UI.s1 cDNA); AA874874 (ESTs, Highly similar to alcohol dehydrogenase class III); AA874969 (ESTs, Highly similar to c-Jun leucine zipper interactive); AA874995 (UI-R-E0-cf-d-08-0-UI.s1 cDNA); AA875004 (UI-R-E0-cb-b-07-0-UI.s1 cDNA); AA875019 (UI-R-E0-cb-f-08-0-UI.s1 cDNA); AA875032 (UI-R-E0-cb-h-09-0-UI.s1 cDNA); AA875037 (UI-R-E0-cb-a-03-0-UI.s1 cDNA); AA875129 (UI-R-E0-bu-e-01-0-UI.s2 cDNA); AA875257 (UI-R-E0-cq-d-12-0-UI.s1 cDNA); AA891037 (EST194840 cDNA); AA891041 (jun B proto-oncogene); AA891221 (EST195024 cDNA); AA891476 (EST195279 cDNA); AA891537 (EST195340 cDNA); AA891690 (ESTs, Weakly similar to p-serine aminotransferase); AA891727 (EST195530 cDNA); AA891734 (EST195537 cDNA); AA891774 (EST195577 cDNA); AA891810 (EST195613 cDNA); AA891880 (Loc65042); AA891916 (membrane interacting protein of RGS16); AA891944 (EST195747 cDNA); AA891950 (EST195753 cDNA); AA892146 (EST195949 cDNA); AA892298 (EST196101 cDNA); AA892414 (EST196217 cDNA); AA892511 (EST196314 cDNA); AA892520 (EST196323 cDNA); AA892538 (EST196341 cDNA); AA892637 (EST196440 cDNA); AA892775 (Lysozyme);

AA892796 (EST196599 cDNA); AA892813 (EST196616 cDNA); AA892986 (EST196789 cDNA); AA893082 (EST196885 cDNA); AA893185 (EST196988 cDNA); AA893199 (EST197002 cDNA); AA893224 (EST197027 cDNA); AA893320 (EST197123 cDNA); AA893584 (EST197387 cDNA); AA893690 (EST197493 cDNA); AA893708 (KIAA0560); AA893717 (EST197520 cDNA); AA893743 (EST197546 cDNA); AA893788 (ESTs, Highly similar to chromobox protein homolog 5); AA893946 (EST197749 cDNA); AA894305 (EST198108 cDNA); AA924925 (ER transmembrane protein Dri 42); AA942685 (cytosolic cysteine dioxygenase 1); AA955306 (ras-related protein rab10); AA955388 (Na^+K^+ transporting ATPase 2, beta polypeptide 2); AA957132 (N-acetylglucosaminyltransferase I); AB000778 (Phoshpolipase D gene 1); AB006451 (Tim23); AB008538 (HB2); AB016532 (period homolog 2 (Drosophila)); AF000899 (p58/p45, nucleolin); AF007554 (Mucin1); AF007758 (synuclein, alpha); AF007890 (resection-induced TPI (rs11)); AF008554 (implantation-associated protein (IAG2)); AF013144 (MAP-kinase phosphatase (cpg21)); AF016269 (kallikrein 6 (neurosin, zyme)); AF016296 (neuropilin); AF019974 (Chromogranin B, parathyroid secretory protein); AF020046 (integrin alpha E1, epithelial-associated); AF021935 (Ser-Thr protein kinase); AF023087 (Early growth response 1); AF030050 (replication factor C); AF030088 (RuvB-like protein 1); AF040954 (putative protein phosphatase1 nuclear targeting subunit); AF051561 (solute carrier family 12, member 2); AF055477 (L-type voltage-dependent Ca^{2+} channel (?1D subunit)); AF074608 (MHC class I antigen (RT1.EC2) gene); AF076183 (cytosolic sorting protein PACS-1a (PACS-1)); AF095927 (protein phosphatase 2C); AI010110 (SH3-domain GRB2-like 1); AI012589 (glutathione S-transferase, pi 2); AI013627 (defender against cell death 1); AI013861 (3-hydroxyisobutyrate dehydrogenase); AI045249 (heat shock 70kD protein 8); AI102031 (myc box dependent interacting protein 1); AI102299 (Bid3); AI102839 (cerebellar Ca-binding protein, spot 35 protein); AI102868 (EST212157 cDNA); AI104388 (heat shock 27kD protein 1); AI136891 (zinc finger protein 36, C3H type-like 1); AI168942 (branched chain keto acid dehydrogenase E1); AI169265 (Atp6s1); AI171966 (ESTs, Highly similar to selenide, water dikinase 2); AI175973 (ESTs, Highly similar to NADH dehydrogenase); AI176491 (EST220076 cDNA); AI176595 (Cathepsin L); AI176621 (iron-responsive element-binding protein);

cDNA clone rx00973 3); AA858620 (UI-R-E0-bq-b-09-0-UI.s1 cDNA); AA866291 (UI-R-A0-ac-e-12-0-UI.s3 cDNA); AA894104 (EST197907 cDNA); AA799996 (EST189493 cDNA); AA892805 (EST196608 cDNA); AI639019 (mixed-tissue library cDNA clone rx01107 3); AA799538 (EST189035 cDNA); AI070108 (UI-R-Y0-lu-a-09-0-UI.s1 cDNA); AA866409 (UI-R-E0-ch-a-03-0-UI.s1 cDNA); AA859632 (UI-R-E0-bs-h-08-0-UI.s1 cDNA); AA891651 (EST195454 cDNA); AA893032 (ESTs, Moderately similar to CALX calnexin precursor); AA891965 (EST195768 cDNA); AA800708 (ESTs, Weakly similar to S28312 hypothetical protein F02A9.4); AA964320 (UI-R-C0-gu-e-09-0-UI.s1 cDNA); AA893173 (EST196976 cDNA); H32977 (EST108553 cDNA); AA874887 (UI-R-E0-ci-g-10-0-UI.s1 cDNA); AA850781 (EST193549 cDNA); AI176456 (ESTs, Weakly similar to endothelial actin-binding protein); H31418 (EST105434 cDNA); AA858588 (ESTs, Weakly similar to ODP2 dihydrolipoamide acetyl transferase); AA891785 (EST195588 cDNA); AA799803 (ESTs, Weakly similar to K1CU cytoskeletal keratin (type 1)); AA799449 (EST, Weakly similar to UBP4 ubiquitin carboxyl-terminal hydrolase 4); AA859777 (UI-R-E0-bu-e-10-0-UI.s1 cDNA); AI639532 (mixed-tissue library cDNA clone rx01030 3); AA875059 (UI-R-E0-cb-f-04-0-UI.s1 cDNA); AI012051 (EST206502 cDNA); AA800549 (EST190046 cDNA); AA799854 (EST189351 cDNA); AA892520 (EST196323 cDNA) and mammalian homologues thereof.

48. The use of claim 47, wherein the assay for aging is a measurement of age-dependent cognitive decline.

49. The use of claim 48, wherein the age-dependent cognitive decline is an age-dependent neurodegenerative condition.

50. The use of claim 49, wherein the age-dependent neurodegenerative condition is Alzheimer's disease or Parkinson's disease.

51. The use of claim 47, wherein the assay for aging is a measurement of the degree of the safety or effectiveness of compounds or procedures directed against age-related cognitive decline.

52. A set of biomarkers for brain aging, comprising mammalian polynucleotides and polypeptides encoded by said polynucleotides:

(a) wherein the set of biomarkers comprises at least two members; and

(b) wherein the brain expression patterns of the members of the set are significantly altered with aging as determined by a conventional statistical method, with $p < 0.05$.

53. The set of biomarkers of claim 52, wherein the set contains at least one member selected from the group consisting of AA685974 (EST108806 cDNA); AA799396 (EST188893 cDNA); AA799479 (ESTs, Highly similar to NADH-ubiquinone oxidoreduct.); AA799481 (EST188978 cDNA); AA799529 (EST189026 cDNA); AA799599 (EST189096 cDNA); AA799636 (EST189133 cDNA); AA799680 (EST189177 cDNA); AA799724 (ESTs, Highly similar to DNA-directed RNA polymeraseI); AA799773 (EST189270 cDNA); AA799779 (acyl-CoA:dihydroxyacetonephosphate acyltransferase); AA799858 (EST189355 cDNA); AA800026 (EST189523 cDNA); AA800318 (EST189815 cDNA); AA800622 (EST190119 cDNA); AA800693 (EST190190 cDNA); AA800948 (Tuba4); AA801286 (Inositol (myo)-1(or 4)-monophosphatase 1); AA817887 (profilin); AA818025 (CD59 antigen); AA818240 (Nuclear pore complex protein); AA818487 (cyclophilin B); AA819500 (ESTs, Highly similar to AC12_HUMAN 37 kD subunit); AA819708 (Cox7a3); AA848831 (lysophosphatidic acid G-protein-coupled receptor, 2); AA852046 (EST194815 cDNA); AA859545 (ESTs, Weakly similar to hypothetical protein C09H6.3); AA859562 (UI-R-E0-bv-b-03-0-UI.s1 cDNA); AA859643 (UI-R-E0-bs-a-08-0-UI.s1 cDNA); AA859645 (attractin); AA859690 (UI-R-E0-bx-e-11-0-UI.s1 cDNA); AA859848 (UI-R-E0-cc-h-10-0-UI.s1 cDNA); AA859954 (Vacuole Membrane Protein 1); AA859980 (T-complex 1); AA860030 (UI-R-E0-bz-e-07-0-UI.s2 cDNA); AA866257 (ESTs); AA866299 (UI-R-A0-ac-f-12-

0-UI.s3 cDNA); AA866299 (UI-R-A0-ac-f-12-0-UI.s3 cDNA); AA866432 (UI-R-E0-ch-e-06-0-UI.s1 cDNA); AA866477 (UI-R-E0-br-h-03-0-UI.s1 cDNA); AA874830 (UI-R-E0-cg-f-04-0-UI.s1 cDNA); AA874874 (ESTs, Highly similar to alcohol dehydrogenase class III); AA874969 (ESTs, Highly similar to c-Jun leucine zipper interactive); AA874995 (UI-R-E0-cf-d-08-0-UI.s1 cDNA); AA875004 (UI-R-E0-cb-b-07-0-UI.s1 cDNA); AA875019 (UI-R-E0-cb-f-08-0-UI.s1 cDNA); AA875032 (UI-R-E0-cb-h-09-0-UI.s1 cDNA); AA875037 (UI-R-E0-cb-a-03-0-UI.s1 cDNA); AA875129 (UI-R-E0-bu-e-01-0-UI.s2 cDNA); AA875257 (UI-R-E0-cq-d-12-0-UI.s1 cDNA); AA891037 (EST194840 cDNA); AA891041 (jun B proto-oncogene); AA891221 (EST195024 cDNA); AA891476 (EST195279 cDNA); AA891537 (EST195340 cDNA); AA891690 (ESTs, Weakly similar to p-serine aminotransferase); AA891727 (EST195530 cDNA); AA891734 (EST195537 cDNA); AA891774 (EST195577 cDNA); AA891810 (EST195613 cDNA); AA891880 (Loc65042); AA891916 (membrane interacting protein of RGS16); AA891944 (EST195747 cDNA); AA891950 (EST195753 cDNA); AA892146 (EST195949 cDNA); AA892298 (EST196101 cDNA); AA892414 (EST196217 cDNA); AA892511 (EST196314 cDNA); AA892520 (EST196323 cDNA); AA892538 (EST196341 cDNA); AA892637 (EST196440 cDNA); AA892775 (Lysozyme); AA892796 (EST196599 cDNA); AA892813 (EST196616 cDNA); AA892986 (EST196789 cDNA); AA893082 (EST196885 cDNA); AA893185 (EST196988 cDNA); AA893199 (EST197002 cDNA); AA893224 (EST197027 cDNA); AA893320 (EST197123 cDNA); AA893584 (EST197387 cDNA); AA893690 (EST197493 cDNA); AA893708 (KIAA0560); AA893717 (EST197520 cDNA); AA893743 (EST197546 cDNA); AA893788 (ESTs, Highly similar to chromobox protein homolog 5); AA893946 (EST197749 cDNA); AA894305 (EST198108 cDNA); AA924925 (ER transmembrane protein Dri 42); AA942685 (cytosolic cysteine dioxygenase 1); AA955306 (ras-related protein rab10); AA955388 (Na^+K^+ transporting ATPase 2, beta polypeptide 2); AA957132 (N-acetylglucosaminyltransferase I); AB000778 (Phoshpolipase D gene 1); AB006451 (Tim23); AB008538 (HB2); AB016532 (period homolog 2 (Drosophila)); AF000899 (p58/p45, nucleolin); AF007554 (Mucin1); AF007758 (synuclein, alpha); AF007890

(resection-induced TPI (rs11)); AF008554 (implantation-associated protein (IAG2)); AF013144 (MAP-kinase phosphatase (cpg21)); AF016269 (kallikrein 6 (neurosin, zyme)); AF016296 (neuropilin); AF019974 (Chromogranin B, parathyroid secretory protein); AF020046 (integrin alpha E1, epithelial-associated); AF021935 (Ser-Thr protein kinase); AF023087 (Early growth response 1); AF030050 (replication factor C); AF030088 (RuvB-like protein 1); AF040954 (putative protein phosphatase1 nuclear targeting subunit); AF051561 (solute carrier family 12, member 2); AF055477 (L-type voltage-dependent Ca^{2+} channel (?1D subunit)); AF074608 (MHC class I antigen (RT1.EC2) gene); AF076183 (cytosolic sorting protein PACS-1a (PACS-1)); AF095927 (protein phosphatase 2C); AI010110 (SH3-domain GRB2-like 1); AI012589 (glutathione S-transferase, pi 2); AI013627 (defender against cell death 1); AI013861 (3-hydroxyisobutyrate dehydrogenase); AI045249 (heat shock 70kD protein 8); AI102031 (myc box dependent interacting protein 1); AI102299 (Bid3); AI102839 (cerebellar Ca-binding protein, spot 35 protein); AI102868 (EST212157 cDNA); AI104388 (heat shock 27kD protein 1); AI136891 (zinc finger protein 36, C3H type-like 1); AI168942 (branched chain keto acid dehydrogenase E1); AI169265 (Atp6s1); AI171966 (ESTs, Highly similar to selenide, water dikinase 2); AI175973 (ESTs, Highly similar to NADH dehydrogenase); AI176491 (EST220076 cDNA); AI176595 (Cathepsin L); AI176621 (iron-responsive element-binding protein); AI177404 (EST221024 cDNA); AI178204 (EST221869 cDNA); AI178921 (Insulin degrading enzyme); AI228548 (ESTs, Highly similar to DKFZp586G0322.1); AI230247 (selenoprotein P, plasma, 1); AI230778 (ESTs, Highly similar to protein-tyrosine sulfotrans. 2); AI230914 (farnesyltransferase beta subunit); AI231807 (ferritin light chain 1); AI231807 (ferritin light chain 1); AI232268 (LDL receptor-related protein associated protein 1); AI235344 (geranylgeranyltransferase type I (GGTase-I)); AI237007 (ESTs, Highly similar to flavoprot.-ubiquin. Oxidoreduct.); AI638971 (mixed-tissue library cDNA clone rx04989 3); AI638997 (mixed-tissue library cDNA clone rx05048 3); AI639151 (mixed-tissue library cDNA clone rx02802 3); AI639209 (mixed-tissue library cDNA clone rx00680 3); AI639257 (mixed-tissue library cDNA clone rx01119 3); AI639477 (mixed-tissue library cDNA clone rx02351 3); D00569 (2,4-dienoyl CoA reductase 1, mitochondrial); D10262

(choline kinase); D10699 (ubiquitin carboxy-terminal hydrolase L1); D10854 (aldehyde reductase); D10874 (lysosomal vacuolar proton pump (16 kDa)); D16478 (mitochondrial long-chain enoyl-CoA hydratase); D17309 (delta 4-3-ketosteroid-5-beta-reductase); D28110 (myelin-associated oligodendrocytic basic protein); D28110 (myelin-associated oligodendrocytic basic protein); D28557 (cold shock domain protein A); D29766 (v-crk-associated tyrosine kinase substrate); D37951 (MIBP1 (c-myc intron binding protein 1)); D45247 (proteasome subunit RCX); D45249 (protease (prosome, macropain) 28 subunit, alpha); D78308 (calreticulin); D83948 (adult liver S1-1 protein); D88586 (eosinophil cationic protein); D89340 (dipeptidylpeptidase III); D89730 (Fibulin 3, fibulin-like extracellular matrix protein 1); D90211 (Lysosomal-associated membrane protein 2); E03229 (cytosolic cysteine dioxygenase 1); H33086 (EST108750 cDNA); H33725 (associated molecule with the SH3 domain of STAM); J02752 (acyl-coA oxidase); J02773 (heart fatty acid binding protein); J05022 (peptidylarginine deiminase); J05031 (Isovaleryl Coenzyme A dehydrogenase); J05132 (UDP-glucuronosyltransferase); K02248 (Somatostatin); L00191 (Fibronectin 1); L13202 (RATHFH2 HNF-3/fork-head homolog-2 (HFH-2)); L19998 (sulfotransferase family 1A, phenol-preferring, member 1); L24896 (glutathione peroxidase 4); L25605 (Dynamin 2); L26292 (Kruppel-like factor 4 (gut)); L29573 (neurotransmitter transporter,noradrenalin); L42855 (transcription elongation factor B (SIII) polypeptide 2); M10068 (NADPH-cytochrome P-450 oxidoreductase); M13100 (long interspersed repetitive DNA sequence LINE3); M13100 (long interspersed repetitive DNA sequence LINE3); M19936 (Prosaposin-sphingolipid hydrolase activator); M23601 (Monoamine oxidase B); M24104 (synaptobrevin 2); M24104 (Vesicle-associated membrane protein (synaptobrevin 2)); M24852 (Neuron specific protein PEP-19 (Purkinje cell protein 4)); M31174 (thyroid hormone receptor alpha); M36453 (Inhibin, alpha); M55015 (nucleolin); M57276 (Leukocyte antigen (Ox-44)); M58404 (thymosin, beta 10); M80550 (adenylyl cyclase); M83745 (Protein convertase subtilisin / kexin, type I); M89646 (ribosomal protein S24); M91234 (VL30 element); M93273 (somatostatin receptor subtype 2); M93669 (Secretogranin II); M99485 (Myelin oligodendrocyte glycoprotein); S53527 (S100 calcium-binding protein, beta (neural)); S61868 (Ryudocan/syndecan 4);

S72594 (tissue inhibitor of metalloproteinase 2); S77492 (Bone morphogenetic protein 3); S77858 (non-muscle myosin alkali light chain); U04738 (Somatostatin receptor subtype 4); U07619 (Coagulation factor III (thromboplastin, tissue factor)); U08259 (Glutamate receptor, N-methyl D-aspartate 2C); U10357 (pyruvate dehydrogenase kinase 2 subunit p45 (PDK2)); U14950 (tumor suppressor homolog (synapse associ. protein)); U17254 (immediate early gene transcription factor NGFI-B); U17254 (immediate early gene transcription factor NGFI-B); U18771 (Ras-related protein Rab-26); U27518 (UDP-glucuronosyltransferase); U28938 (receptor-type protein tyrosine phosphatase D30); U38379 (Gamma-glutamyl hydrolase); U38801 (DNA polymerase beta); U67080 (r-MyT13); U67136 (A kinase (PRKA) anchor protein 5); U67137 (guanylate kinase associated protein); U72620 (Lot1); U75405 (procollagen, type I, alpha 1); U75917 (clathrin-associated protein 17); U77777 (interleukin 18); U78517 (cAMP-regulated guanine nucleotide exchange factor II); U89905 (alpha-methylacyl-CoA racemase); V01244 (Prolactin); X02904 (glutathione S-transferase P subunit); X05472 (2.4 kb repeat DNA right terminal region); X06769 (FBJ v-fos oncogene homolog); X06916 (S100 calcium-binding protein A4); X13905 (ras-related rab1B protein); X14323 (Fc receptor, IgG, alpha chain transporter); X16933 (RNA binding protein p45AUF1); X53427 (glycogen synthase kinase 3 alpha (EC 2.7.1.37)); X53504 (ribosomal protein L12); X54467 (cathepsin D); X55153 (ribosomal protein P2); X57281 (Glycine receptor alpha 2 subunit); X58294 (carbonic anhydrase 2); X59737 (ubiquitous mitochondrial creatine kinase); X60212 (ASI homolog of bacterial ribosomal subunit protein L22); X62950 (pBUS30 with repetitive elements); X67805 (Synaptonemal complex protein 1); X72757 (cox VIa gene (liver)); X74226 (LL5 protein); X76489 (CD9 cell surface glycoprotein); X76985 (latexin); X82445 (nuclear distribution gene C homolog (Aspergillus)); X84039 (lumican); X89696 (TPCR06 protein); X97443 (integral membrane protein Tmp21-I (p23)); X98399 (solute carrier family 14, member 1); Y17295 (thiol-specific antioxidant protein (1-Cys peroxiredoxin)); Z48225 (protein synthesis initiation factor eIF-2B delta subunit); Z49858 (plasmolipin) and mammalian homologues.

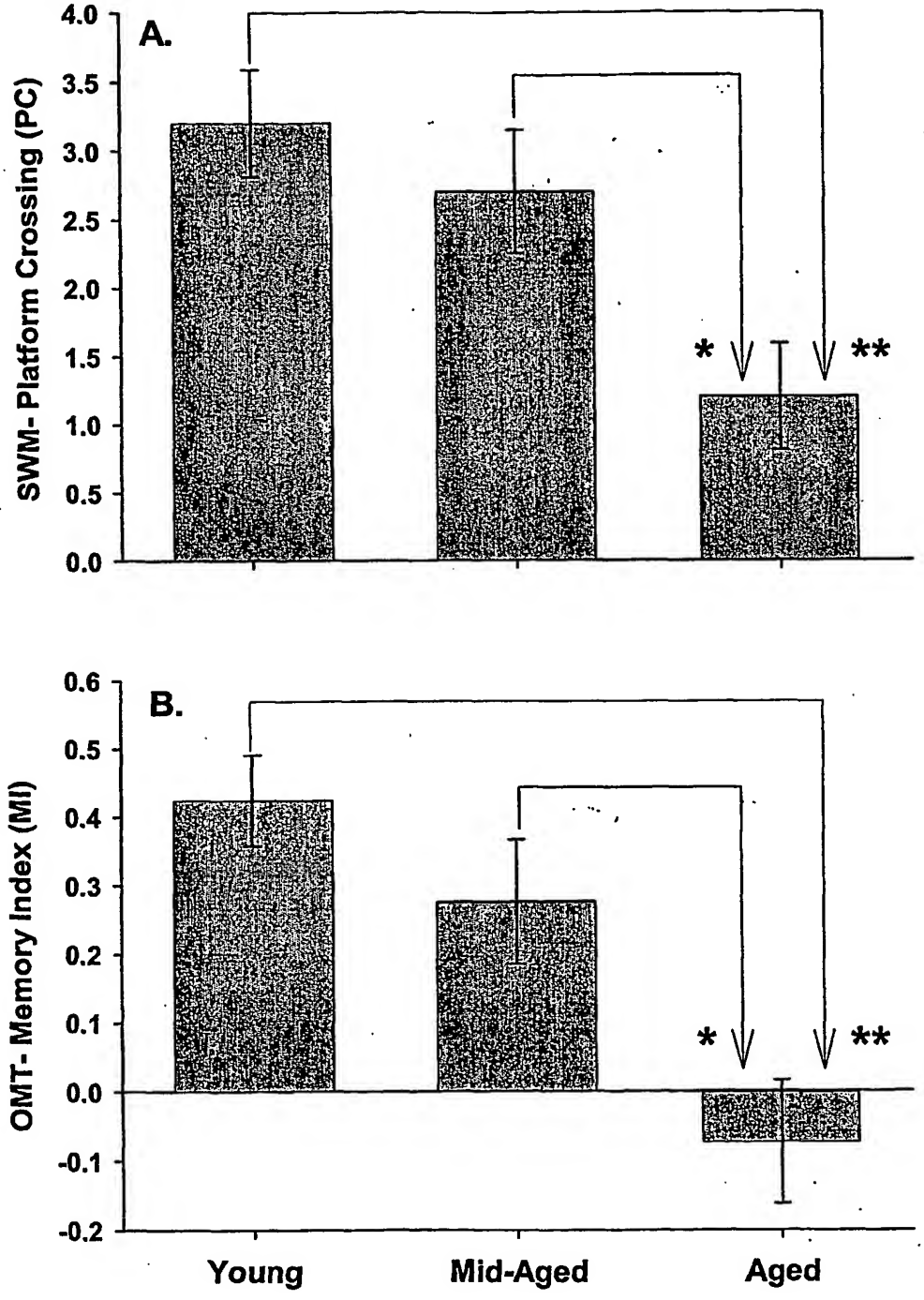

A.
SWM- Platform Crossing (PC)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
**
B.
OMT- Memory Index (MI)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
*
**
Young
Mid-Aged
Aged

FIG. 1

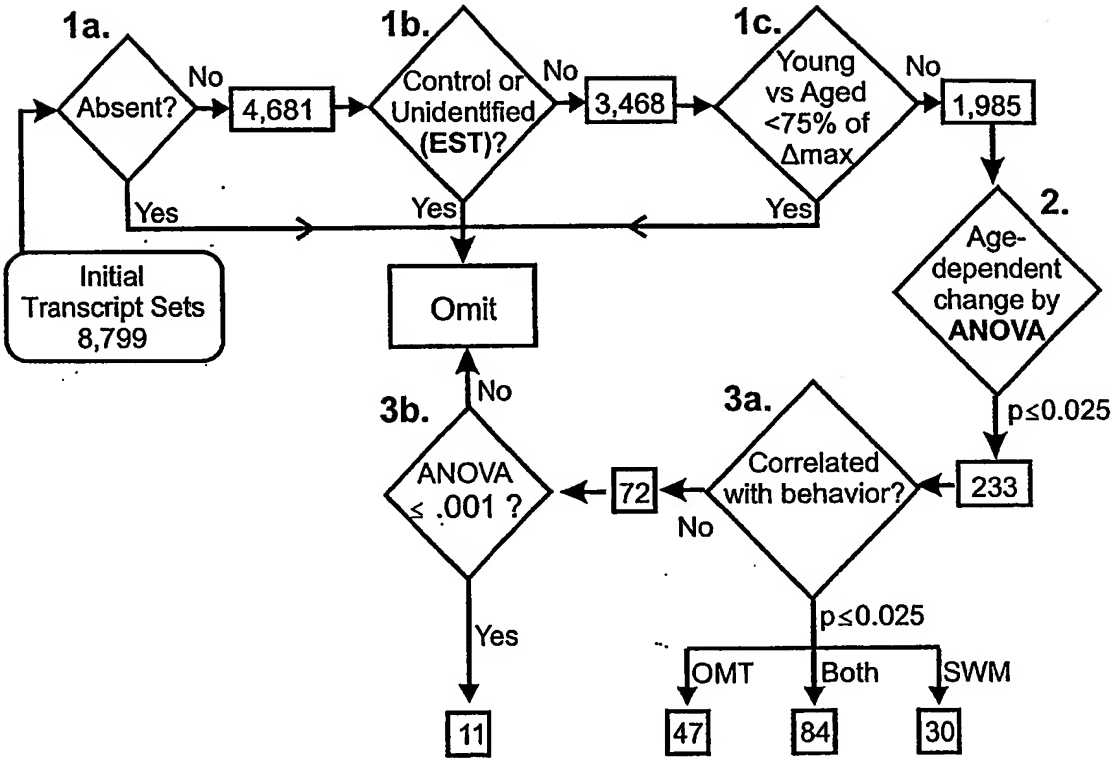
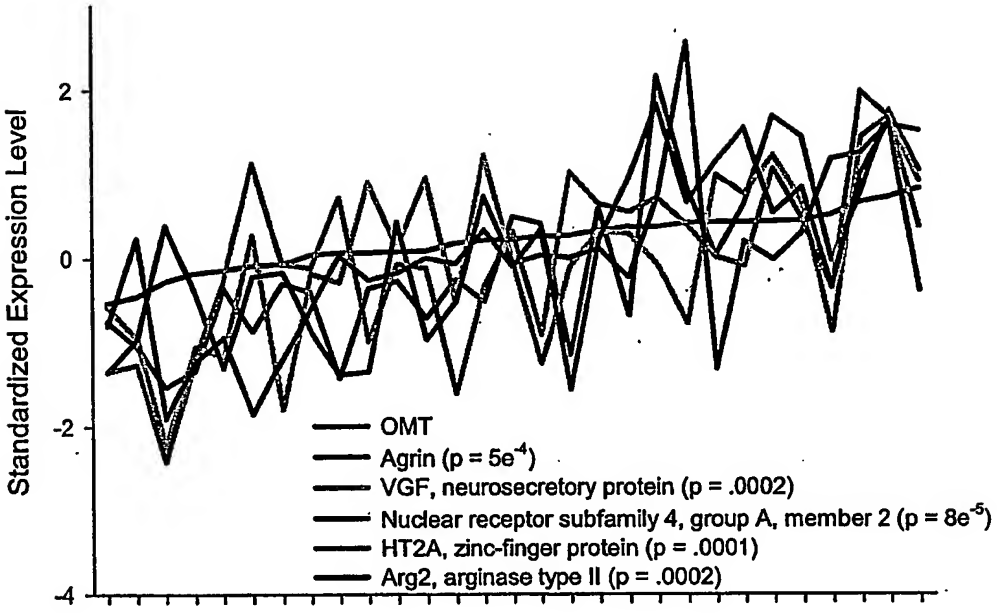

1a.
Absent?
No
4,681
1b.
Control or Unidentified (EST)?
No
3,468
1c.
Young vs Aged <75% of Δmax
No
1,985
2.
Age-dependent change by ANOVA
p≤0.025
233
3a.
Correlated with behavior?
No
72
3b.
ANOVA ≤ .001 ?
No
Yes
11
p≤0.025
OMT
47
Both
84
SWM
30
Yes
Yes
Yes
Initial Transcript Sets 8,799
Omit

FIG. 2

A.


Standardized Expression Level
2
0
-2
-4
OMT
Agrin (p = 5e-4)
VGF, neurosecretory protein (p = .0002)
Nuclear receptor subfamily 4, group A, member 2 (p = 8e-5)
HT2A, zinc-finger protein (p = .0001)
Arg2, arginase type II (p = .0002)

B.

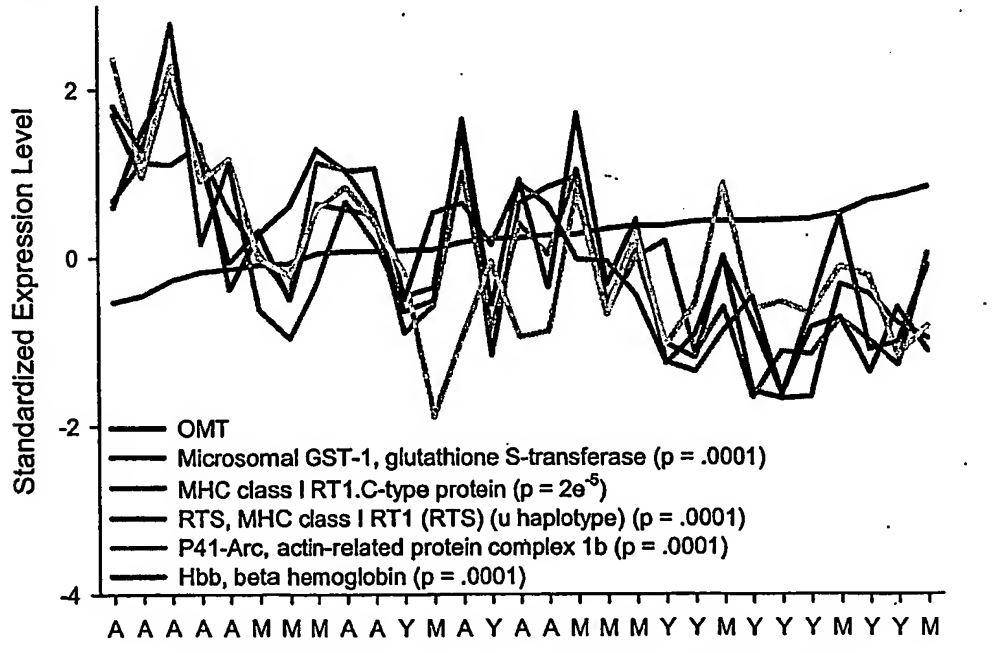

Standardized Expression Level
2
0
-2
-4
OMT
Microsomal GST-1, glutathione S-transferase (p = .0001)
MHC class I RT1.C-type protein (p = 2e-5)
RTS, MHC class I RT1 (RTS) (u haplotype) (p = .0001)
P41-Arc, actin-related protein complex 1b (p = .0001)
Hbb, beta hemoglobin (p = .0001)
A A A A A M M M A A Y M A Y A A M M M Y Y M Y Y Y M Y Y M
Animals Ranked from Lowest to Highest Performance
(A = Aged, M = Mid Aged, Y = Young)

FIG. 3

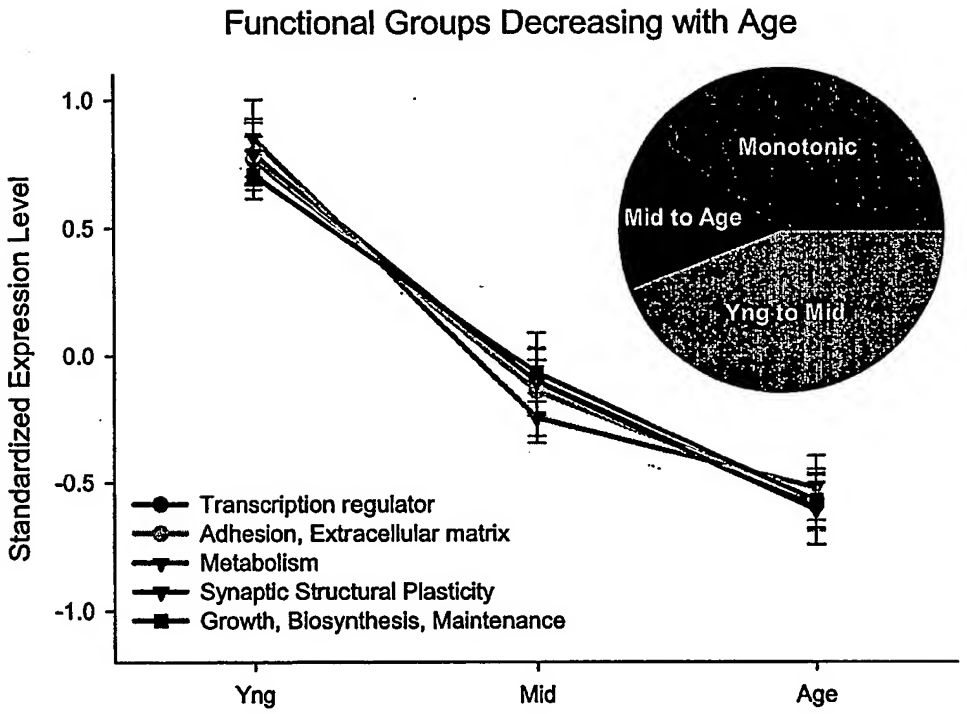

Functional Groups Decreasing with Age
Standardized Expression Level
1.0
0.5
0.0
-0.5
-1.0
Yng
Mid
Age
Monotonic
Mid to Age
Yng to Mid
Transcription regulator
Adhesion, Extracellular matrix
Metabolism
Synaptic Structural Plasticity
Growth, Biosynthesis, Maintenance

FIG. 4

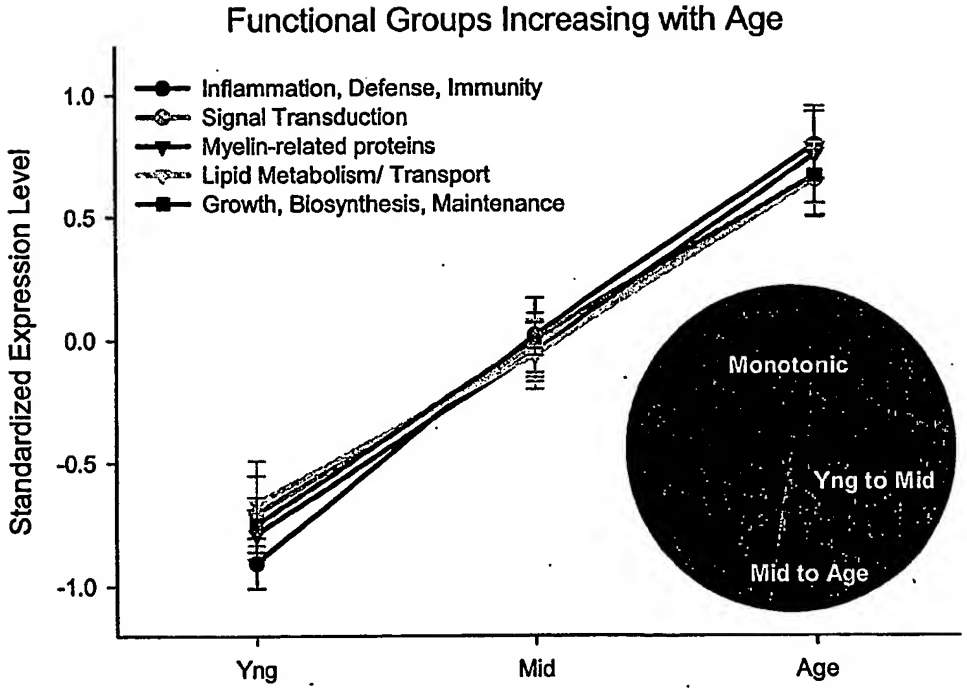

Functional Groups Increasing with Age
Inflammation, Defense, Immunity
Signal Transduction
Myelin-related proteins
Lipid Metabolism/ Transport
Growth, Biosynthesis, Maintenance
Standardized Expression Level
1.0
0.5
0.0
-0.5
-1.0
Yng
Mid
Age
Monotonic
Yng to Mid
Mid to Age

FIG. 5

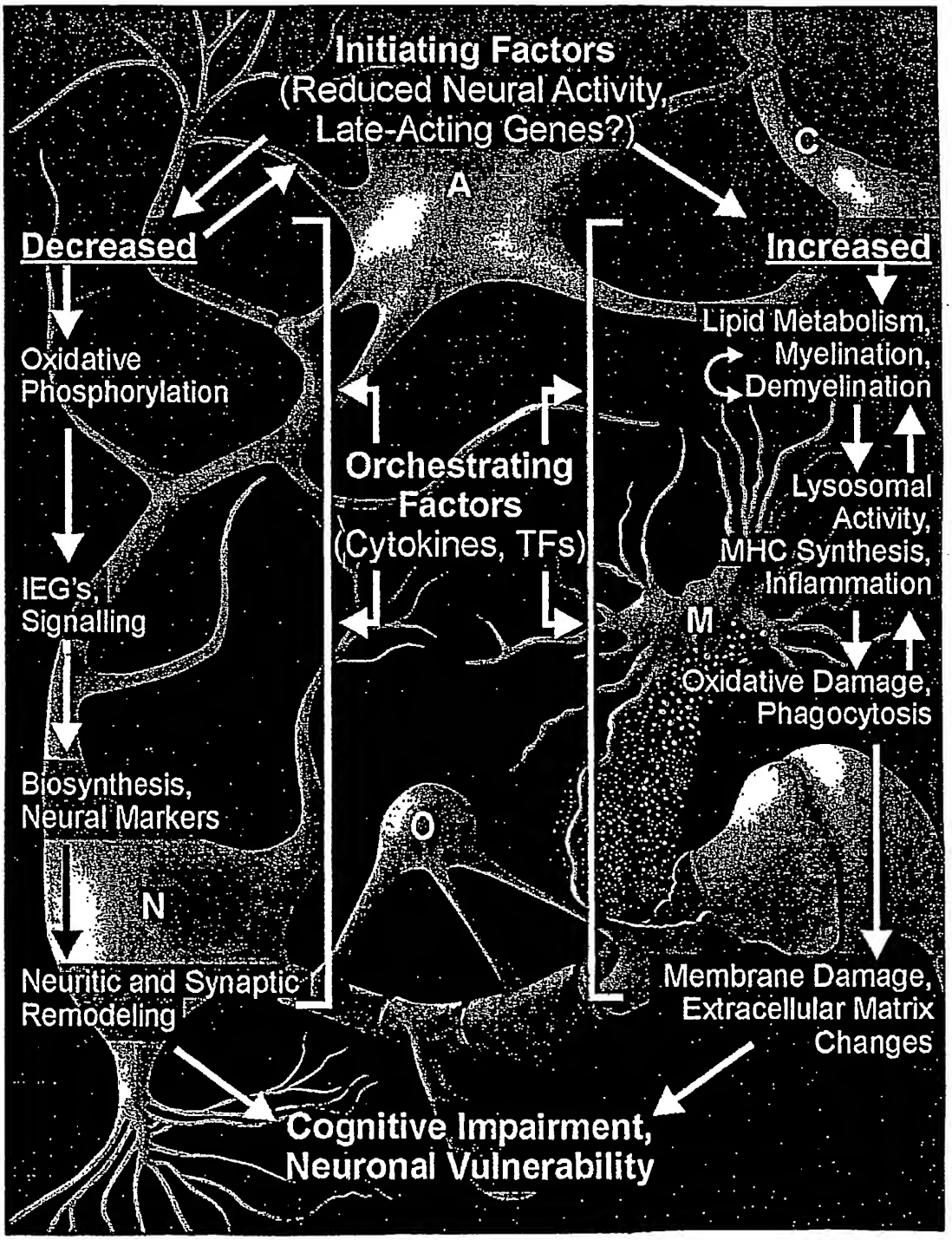

Initiating Factors
(Reduced Neural Activity,
Late-Acting Genes?)
A
C
Decreased
Increased
Oxidative
Phosphorylation
Lipid Metabolism,
Myelination,
Demyelination
Orchestrating
Factors
(Cytokines, TFs)
Lysosomal
Activity,
MHC Synthesis,
Inflammation
IEG's,
Signalling
M
Oxidative Damage,
Phagocytosis
Biosynthesis,
Neural Markers
O
N
Neuritic and Synaptic
Remodeling
Membrane Damage,
Extracellular Matrix
Changes
Cognitive Impairment,
Neuronal Vulnerability

FIG. 6

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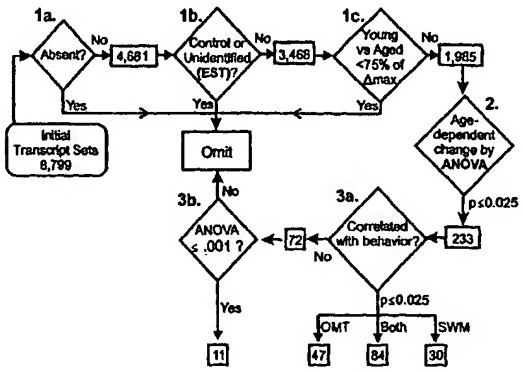
[Continued on next page]

(54) Title: GENE EXPRESSION PROFILE BIOMARKERS AND THERAPEUTIC TARGETS FOR BRAIN AGING AND AGE-RELATED COGNITIVE IMPAIRMENT



(57) Abstract: A statistical and functional correlation strategy to identify changes in cellular pathways specifically linked to impaired cognitive function with aging. Analyses using the strategy identified multiple groups of genes expressed in the hippocampi of mammals, where the genes were expressed at different levels for several ages. The aging changes in expression began before mid-life. Many of the genes were involved in specific neuronal and glial pathways with previously unrecognized relationships to aging and/or cognitive decline. The processes identified by the strategy suggest a new hypothesis of brain aging in which initially decreased neuronal activity and/or oxidative metabolism trigger separate but parallel genomic cascades in neurons and glia. In neurons, the cascade results in elevations in calcium signaling and reductions of immediate early gene signaling, biosynthesis, synaptogenesis and neurite remodeling. In contrast, glia undergo increased lipid metabolism and mediate a cycle of demyelination and remyelination that induces antigen presentation, inflammation, oxidative stress and extracellular restructuring. These identified genes and the proteins they encode can be used as novel biomarkers of brain aging and as targets for developing treatment methods against age-related cognitive decline, Alzheimer's Disease and Parkingson's Disease.

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Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

GENE EXPRESSION PROFILE BIOMARKERS AND THERAPEUTIC TARGETS FOR BRAIN AGING AND AGE-RELATED COGNITIVE IMPAIRMENT

STATEMENT OF GOVERNMENT INTEREST

[01] This invention has been made in part with government support under grants AG04542, AG10836, AG18228 and AG14979 from the National Institute on Aging, and by MH59891. The government of the United States of America may have certain rights in this invention.

FIELD OF THE INVENTION

[02] The invention relates generally to genetic algorithms, and more particularly to the identification of gene expression profile biomarkers and therapeutic targets for brain aging.

BACKGROUND OF THE INVENTION

[03] Brain aging processes are enormously complex phenomena that affect multiple systems, cell types and pathways, and result in cognitive decline and increased risk of Alzheimer's disease (AD). Landfield PW *et al., J Neurobiol 23*: 1247-1260 (1992). Although several biological mechanisms have been putatively linked to brain aging or Alzheimer's disease, including inflammation, oxidative stress, Ca^{2+} dyshomeostasis (Landfield, PW & Pitler TA, *Science 226*: 1089-1092 (1984); Landfield PW *et al., J Neurobiol 23*: 1247-1260 (1992)), mitochondrial dysfunction and chronic exposure to adrenal stress hormones (Landfield PW *et al., Science 214*: 581-584 (1981); Porter NM & Landfield PW, *Nature Neurosci 1*: 3-4 (1998)), the specific mechanisms and pathways, if any, through which they are linked to impaired brain function are not understood.

[04] It is widely thought that gene expression changes contribute to many aspects of declining function with aging. Finch CE, *Longevity, Senescence and the Genome,* 37-42 (Univ. Chicago Press, Chicago, 1990). It is also thought that gene expression changes are important for processing and storage of memory. However, not all genes that change expression in the brain with aging are thought to be important for cognition.

[05] Gene-expression changes that specifically contribute to age-related memory decline should selectively change with brain aging and should be correlated specifically with measures of age-associated cognitive decline; that is, a subset of the full set of

aging-dependent genes should also correlate with age-related cognitive decline. *See*, Lockhart DJ & Barlow C, *Nat Rev Neurosci 2:* 63-68 (2001) and Mirnics K, *Nat Rev Neurosci 2:* 444-447 (2001).

[06] If a subset of age-dependent genes also shows expression patterns directly correlated with age-related memory decline, then such a subset of "aging and cognition-related genes" (ACGs) would be extremely helpful as biological indexes ("biomarkers") for assessing or diagnosing the degree of age-related cognitive impairment in individual subjects. In turn, the ability to measure aging-related cognitive impairment quantitatively is essential for discovering new therapeutic targets, and developing new strategies and pharmaceutical compounds for counteracting normal age-related cognitive decline and/or age-related neurodegenerative diseases, including Alzheimer's disease (AD) or Parkinson's disease (PD).

[07] Identifying ACGs in any mammalian species therefore, might have great therapeutic usefulness. Moreover, because of the well-established homologies of most genes across mammalian species and because of the clear similarities in patterns of brain aging and cognitive decline across species, identification in any mammal would have human health implications. Furthermore, because the primary risk factor for Alzheimer's disease and Parkinson's disease is aging itself, therapeutic approaches developed for aging-related cognitive impairment should also help ameliorate cognitive decline from age-related neurodegenerative disease. Thus, there is a clear need for identifying ACGs but, to date, such genes have not been discovered for any mammal.

[08] Gene microarray technology provides a powerful approach for unraveling the complex processes of aging. To date, however, its impact has been limited by statistical problems, small sample sizes, and difficulty in assessing functional relevance. Moreover, studies that have examined gene expression during brain aging using microarrays have not used sample sizes large enough to provide adequate statistical power for formal statistical testing. Lee CK *et al., Nature Genetics 25*: 294-297 (2000); Jiang CH *et al., Proc Natl Acad Sci USA 98*: 1930-1934 (2001) Therefore, even the genes they have reported to change with aging have not been validated by accepted statistical criteria.

[09] The extremely large data sets generated by microarrays pose formidable bioinformatics and resource problems that have to date limited the impact of this powerful technology. Because of these difficulties, most microarray studies have relied on simple fold change comparisons in small samples. However, neither fold change analyses nor the small

sample protocols widely used allow the direct estimates of variance necessary for defining type I error (false positives). In addition, fold change criteria, by definition, select for large changes. Therefore, they exhibit low detection sensitivity (high false negatives, or type II error), and are unable to identify the modest changes that often characterize functionally important (and, therefore, tightly regulated) genes. The inability to assign type I error is a particularly critical problem for microarray studies because the thousands of comparisons of gene expression in such analyses greatly increase the expected false positives. For example, even if group sizes were sufficient for formal statistical analyses, and 5000 gene transcripts were each tested by *t-test* for differences between two conditions at $p \leq 0.05$, the false positive rate is equal to the *p-value* and, consequently, 5% of the 5000 tested transcripts (250) would be expected to be found significant by chance alone.

[10] Although microarray studies have some important offsetting advantages that improve statistical confidence (*e.g.*, co-regulation of genes within a functional group), there is increasing recognition that microarray experiments should generally meet the same statistical standards as other biological experiments or, at least, should systematically estimate the degree of statistical uncertainty. Several strategies to improve statistical confidence have been developed for small-sample microarray studies, but these generally rely on indirect estimates of variance and/or greatly sacrifice sensitivity (*i.e.*, stringent *p-values*).

[11] Another highly important problem of microarray studies is that of determining which of the hundreds of expression changes that may be observed are likely to be functionally relevant. Correlation analysis is one quantitative approach to linking gene expression with function, although it also requires relatively large sets of independent samples. Expression-function correlations fulfill a key prediction of a causal relationship (*i.e.*, that causally related variables should co-vary) and therefore, can serve as a valuable tool for the identification of candidate functionally relevant genes. Nonetheless, there have been few correlation studies attempting to link cognitive dysfunction with univariate gene expression patterns across individual subjects, much less using the massive amounts of data generated in microarray analyses.

SUMMARY OF THE INVENTION

[12] The invention provides a statistical and functional correlation strategy to identify changes in cellular pathways specifically linked to impaired cognitive function with aging.

The bioinformatics and functional correlation strategy improves the power of microarray analyses and provides the ability to test whether alterations in specific hippocampal pathways are correlated with aging-related cognitive impairment. The invention is useful for application in large, well-powered groups and for controlling type I error (false positives), enhancing detection sensitivity (reducing type II false negatives) and determining which aging changes in expression are most closely correlated with declining brain function.

[13] Accordingly, the invention provides a method for identifying a biomarker for brain aging, where the biomarker is a polynucleotide or a polypeptide encoded by said polynucleotide. The method involves first obtaining a set of polynucleotides obtained from a set of brain samples (such as hippocampal samples), where the members of the set of brain samples were obtained from members of a set of mammals, wherein the set of mammals contains more than two members, with at least young, mid-aged and aged members, and then identifying the identity and amount of the members of the set of polynucleotides present in the brain samples. The method then involves the steps of deleting certain non-biomarker polynucleotides from the set of polynucleotides, testing by a conventional statistical method (such as) for a significant effect of aging across the young, mid-aged and aged members; and correlating the identity and amount of the members of the set of polynucleotides present in the brain samples with cognitive performance in behavioral tests.

[14] By use of the methods of the invention, one skilled in the genomics art can identify multiple groups of related genes, many representing processes with previously unrecognized relationships to aging and/or cognitive dysfunction. Thus, the invention also provides compositions of matter comprising sets of genes, expressed sequence tags (ESTs), polynucleotides and polypeptides encoded by said polynucleotides identified as being involved in the aging processes. These sets usefully result in a statistically validated, comprehensive overview of mammalian, including human, functional brain aging. In particular, the set of genes can be used for the diagnosis of human age-related disease, such as an age-related neurodegenerative condition, including Alzheimer's disease or Parkinson disease.

[15] The invention provides a set of biomarkers for brain aging, where (a) the set of biomarkers comprises at least two members; (b) the brain expression patterns of the members of the set are significantly altered with aging as determined by a conventional statistical method (such as ANOVA or student's t test), with $p < 0.05$; (c) the brain expression patterns

of the members of the set are correlated (using a conventional statistical correlation test, *e.g.*, tested by Pearson's or Spearman's correlation test) across age groups with cognitive performance in behavioral tests, with a correlation of $p < 0.05$ (or with a more stringent correlation of $p < 0.01$ or $p < 0.001$) between brain expression and cognitive performance; and (d) the cognitive performance in behavioral tests significantly altered with aging as determined by a conventional statistical method. The biomarkers may also correlate with a behavioral measure of functional impairment, such as an age-related neurodegenerative condition, including Alzheimer's disease or Parkinson's disease.

[16] The invention also provides a set of at least two biomarkers for brain aging, where where the brain expression patterns of the members of the set are significantly altered with aging as measured by a conventional statistical correlation test at a significance level of $p < 0.01$.

[17] The invention further provides a set of at least two biomarkers for brain aging, where the brain expression patterns of the members of the set are significantly altered with aging as determined by a conventional statistical method, with $p < 0.05$ (or a more stringent correlation, such as $p < 0.025$, $p < 0.01$ or $p < 0.001$).

[18] In one example of the invention, rats in three age groups (Young, Mid-Aged, Aged) were characterized on two memory tasks and each mammal's hippocampal CA1 region was analyzed by a microarray analysis for gene expression. These analyses identified multiple groups of genes, many representing pathways with previously unrecognized relationships to aging and/or cognitive decline. The analysis showed that for all groups, the aging changes in expression began by mid-life.

[19] In one aspect of the invention, the known interactions of the identified processes suggest an integrative model of specific cellular cascades that begin in mid-life and eventually impair cognitive function and increase neuronal vulnerability. Initially decreased neuronal activity and/or oxidative metabolism trigger separate but parallel genomic cascades in neurons and glia. In neurons, the cascade results in reductions of immediate early gene signaling, biosynthesis, synaptogenesis and neurite remodeling. In contrast, glia undergo increased lipid metabolism and mediate a cycle of demyelination and remyelination that induces antigen presentation, inflammation, oxidative stress and extracellular restructuring. Intervention studies based on these findings can identify the cause and effect interactions among the complex processes of brain aging.

BRIEF DESCRIPTION OF THE DRAWINGS

[20] FIG. 1 is a set of bar graphs showing age-dependent impairment of memory performance. Male Fischer 344 rats aged 4 months (Young, n = 10), 13 months (Mid-Aged, n = 10) and 24 months (Aged, n = 10) were used. Aged animals exhibited significantly reduced performance on 24 hr memory retention on both the Morris spatial water maze task (SWM; FIG. 1A) and object memory task (OMT; FIG. 1B) in comparison to either Young or Mid-Aged animals (*p<.05, **p<.01, by 1-way ANOVA and Tukey's *post-hoc*). As shown in the bar graph, the Young and Mid-Aged animals did not differ significantly from each on either the SWM or OMT task. On the SWM task (FIG. 1A), higher platform crossings reflects greater retention of the spot where the platform was previously located. For the OMT (FIG. 1B), a higher memory index reflects greater retention of the previously explored object, and resultant increased exploration of the novel object.

[21] FIG. 2 is a flow chart for a filtering and statistical test algorithm for identifying primary set of ACGs. The flow chart also includes the results for an example of the invention. An initial set of 8,799 transcript sets contained on the U34A Gene Chip (*see*, EXAMPLE 2) was filtered prior to statistical testing, to reduce expected false positives. Probe sets were removed if they were called "absent" (1a.), if they were unknown expressed sequence tags (ESTs) (1b.) or if the difference between the Young and Aged groups did not comprise at least 75% of the maximal normalized age differences (1c.). Each of the remaining 1,985 transcript (gene) sets was then tested by ANOVA across the three age groups (n=9-10) to determine if it changed significantly with aging (2.). Each of the 233 genes that changed significantly with age ($p \leq 0.025$) was then tested across all animals (n=29) for significant behavioral correlation with OMT, SWM, or both SWM and OMT (Pearson's; 3a). Furthermore, of the genes that did not correlate with behavior, ones that showed an ANOVA p value $\leq .001$ were also retained for further analysis (3b). In total, 172 genes were considered, 161 of which could be considered ACGs.

[22] FIG. 3 is a set of line graphs showing correlation of gene expression and OMT across individual animals. Behavioral correlation is measured across all age groups. For genes that decreased with aging, the five best positive correlations (A) and for genes that increased with aging, the five best negative correlations (B) are shown (see Legend: correlation p-values in parentheses). Standardized values for both expression and OMT performance are shown on

the Y-axis. The animals were ranked for OMT performance on the X-axis, from worst (1) to best (29), and OMT performance was plotted as a heavy black line on both A and B for the purposes of comparison. Genes involved in early responses and synaptic remodeling were among the five most highly correlated genes that decreased with aging, whereas those related to actin assembly and inflammation were among the five most highly correlated genes that increased with aging.

[23] FIG. 4 is a line graph and pie chart insert showing functional categories and age course of genes decreased with aging. Chronological patterns are shown for aging changes for five of the eight functional categories (some categories were omitted to improve legibility and because they were highly similar to the ones already depicted). Each gene's expression was normalized prior to calculating category mean values. Note that most downregulated categories exhibited ≥50% of mean changes by the Mid-Aged point, and showed relatively less change between the Mid-Aged and Aged animals. No category showed a predominantly Mid-to-Aged pattern of change. The pie-chart insert shows proportion of genes that followed each of the three possible routes to decreased expression with aging.

[24] FIG. 5 is a line graph and pie chart insert showing functional categories and age course of genes increased with aging. Chronological patterns are shown for aging changes for five of the eleven functional categories of behaviorally-correlated upregulated genes (some categories were omitted to increase legibility and because their pattern of change with age was highly similar to that of categories already depicted). Calculations and nomenclature as in FIG. 4. Note that, in contrast to the majority of downregulated genes (FIG. 4), changes in upregulated categories did not tend to level off after mid-life but instead showed continuing change between mid-life and late-life (*e.g.*, a monotonic pattern). Similar patterns were seen when all upregulated genes are considered (Pie-chart inset).

[25] FIG. 6 is a micrograph showing a model of parallel neuronal and glial cascades leading to functional impairment. Early in mid-life, initiating factors (*e.g.*, reduced neuronal activity, onset of late-acting gene expression) induce downregulation of neuronal (N) oxidative phosphorylation triggering a cascade of impaired IEG signalling, biosynthetic potential, and critically, decreased capacity for neurite remodeling and synaptogenesis. In parallel, enhanced lipid metabolism and demyelination are triggered in oligodendrocytes (O) by altered energy metabolism or neural activity. In turn, astrocytes (A) hypertrophy and increase glycolysis of the glucose taken up by astrocytic endfeet on capillaries (C).

Simultaneously, phagocytosis of myelin fragments triggers oxidative damage and inflammatory responses in microglia (M). Eventually, the combined effects of reduced synaptic remodeling, decreased activity and axon conduction, altered extracellular matrix and expanding inflammation result in cognitive failure and neuronal vulnerability.

DETAILED DESCRIPTION OF THE INVENTION

[26] The concept of "biomarker" is well-known and useful concept for those of skill in the genomic art. In general, a biomarker is a measurable biological manifestation that is a quantitative indication of the presence and degree of an underlying biological process of interest.

[27] We have devised a multi-stage method for the identification of biomarkers for brain aging, using gene expression microarrays and behavioral testing. The method of the invention allows one skilled in the genomics art to identify both "aging and cognition-related genes" (ACGs) and unique genes that change with brain aging alone, based on formal statistical testing.

[28] As used in this specification, the word "cognitive" is defined as comprising the higher order intellectual/brain processes involved in learning, including attention, acquisition, short-term memory, long-term memory and memory retrieval, among others.

[29] As used in this specification, across different mammalian species, age definitions are as follows: "Young" mammals are those at or beyond reproductive maturity for the species. "Mid-aged" is defined in two ways: at or around half the average lifespan for the species and at or around the midpoint between reproductive maturity and average lifespan. "Aged" mammals are those at or around average lifespan. Animals intermediate between two ages could be considered as part of the group to which they are most closely chronologically related (with the exception of young animals, for whom it would be inappropriate to include prepubescent individuals)

[30] We used the bioinformatics and functional correlation strategy of the invention for microarray analyses. As a result, we were able to detect multiple groups of related genes that were altered by brain aging and also correlated with cognitive function across individual subjects. Most of the shifts in genomic regulation began by mid-life, well before the onset of measurable cognitive impairment, implying that cognitive function is not altered substantially

without further progression and/or the cumulative effects of the initial changes in gene regulation.

[31] This analysis depended on a novel combination of three approaches for microarray research: (a) the quantitative measurement of the dependent function of interest (cognitive performance), which provided a basis for large-scale expression-function correlation analyses; (b) the application of formal statistical analyses (ANOVA, Pearson's) to large groups of independent microarray samples, which conferred substantial statistical power and high detection sensitivity for even modest changes (low false negative type II error); and c) systematic estimates of the maximum probabilities of false positives in our data. Our results using the method of the invention provide a generally comprehensive overview of hippocampal genes/processes that are altered with brain aging and closely linked to brain functional decline.

[32] To verify the method of the invention, we first tested young (3-4 months old), mid-aged (12-13 months old) and aged (24-25 months old) rats (n = 9-10 per group) for performance on the Morris spatial water maze (SWM) and object memory task (OMT). Both behavioral tests clearly and reliably (statistically) revealed aging-related cognitive impairment (FIG. 1).

[33] We then anesthetized (for euthanasia) all animals and dissected out a region of the brain (CA1 region of the hippocampus) known to be important for memory. These brain tissues were then prepared for analyses of gene expression profiles (mRNA content) on Affymetrix GeneChip microarrays specific for the rat genome (RG-U34A arrays) (one array for each individual rat sample). The microarrays were then read and analyzed for expression profile data on an Affymetrix GeneChip System according to the manufacturer's instructions.

[34] The behavioral and microarray methods that were used can reasonably be expected to apply as well to mice as to rats. Similar behavioral and microarray methods known to those of skill in the art can be used for testing of other mammals, including humans. The utility of the method of the invention for human testing is discussed below.

[35] We then transferred the data into standard computer spreadsheets (*e.g.*, Excel) for performing statistical analyses of the effects of aging. Using Analysis of Variance (ANOVA) we defined the set of genes whose degree of expression changed significantly with brain aging. We then used that set of "Aging Genes" and tested each gene's expression profile (across only the aged animals) for significant correlation with memory performance on the

Object Memory Task (OMT) as well as the Morris spatial water maze (SWM). The "Aging Genes" whose expression patterns correlated significantly with cognitive performance were defined as the primary subset of "Aging and Cognition-Related Genes" (ACGs), and subcategorized as OMT-associated, SWM-associated, or both OMT and SWM-associated. We further included genes with no behavioral association that had an ANOVA p value $\leq .001$ since genes identified at this more stringent level are less subject to the error of multiple testing (FIG. 2, TABLES 1A and 1B).

[36] Based on those large-scale studies, we have developed a list of ACGs that appear to have considerable potential importance for assessing and generating new treatments for age-dependent functional decline (TABLES 1A and 1B).

[37] These lists contain some genes that were identified previously as being linked to brain aging or neurodegeneration (*e.g.*, inflammation or mitochondrial genes, Lee CK *et al., Nature Genetics 25*: 294-297 (2000)) but none has been previously shown to be specifically associated with both brain aging and aging-dependent cognitive impairment. Further, many genes on our list have not even been shown previously to be linked to brain aging alone or to cognition alone. Thus, our lists of ACGs are unique and useful biomarkers and therapeutic targets specifically for aging-dependent cognitive impairment. In addition, our list of all genes that change with brain aging contains many genes never before reported to change with brain aging, and therefore provides a useful and unique panel of gene biomarkers and therapeutic targets for study and treatment of brain aging.

[38] In addition to these lists for identified genes, we have also performed the same analyses and compiled the same lists for unidentified expressed sequence tags (ESTs) that are on the same Affymetrix Chips (TABLE 2). These are valuable data, because once the ESTs are identified, they can provide therapeutic targets.

[39] Using the method of the invention, we were able to identify a number of processes and pathways that previously have not been clearly associated with normal brain aging. The most unexpected findings included altered expression profiles suggestive of increased myelin and lipid turnover, as well as widespread changes indicating coordinated downregulation of oxidative metabolism, decreased neurite outgrowth and synaptogenesis. Other novel genes we identified appear to suggest alterations in general metabolic and biosynthetic chaperone functions. In addition, many of the identified groups confirmed previously described changes in expression for genes regulating several major processes (*e.g.*, inflammation, glial

reactivity, oxidative stress). However, our results also extend the earlier findings considerably by revealing the extent of the changes and the concurrent upregulation of potentially orchestrating transcription factors and cytokines that may provide important clues to pathogenic mechanisms.

[40] In order to begin to develop an integrative overview of potential interactions among the multiple altered expression patterns observed here, we considered functional implications at the pathway level. Our interpretations rely on the functions that have been previously associated with many of the genes identified by those of skill in the genomics art. These are identified through PubMed literature searches, annotations provided by Affymetrix, entries in the SwissProtein database <http://www.expasy.ch/sprot/sprot-top.html> and associations reported in the Genome Ontology (GO; <http://www.geneontology.org>). We also rely on the general assumption held by those of skill in the genomics art that similar changes in the expression of multiple genes of a particular pathway imply like changes in the functions mediated by the encoded proteins of that pathway. Gene expression changes also can reflect compensatory negative feedback regulation (or other dissociations of gene expression and protein function), but the potential confound of dissociation is presumably less of a problem in microarray analyses in which multiple genes in a pathway are observed to change in the same direction. Some of the primary metabolic pathways and processes considered in the interpretations are depicted in TABLE 1.

[41] *Functional Groups.* We found age-dependent upregulation of many ACGs involved in inflammatory/immune/stress responses and downregulation of many involved in energy metabolism. In addition, we found alterations of gene expression reflecting multiple categories/pathways not previously recognized to change with normal aging. These included upregulation of genes for myelin proteins, cholesterol biosynthesis and transport, amino acid metabolism, intracellular Ca^{2+} signaling, and protein processing, as strongly suggesting an ongoing cycle of remyelination and demyelination. We also found widespread downregulation of genes for biosynthesis, immediate early responses, and synaptic structural plasticity, suggestive of neuronal involution. Multiple transcriptional regulators and cytokines were also identified that may play orchestrating roles. Nearly all expression changes began by mid-life but cognition was not impaired until late life. Upregulated genes for inflammation and intracellular Ca^{2+} release were among those most closely correlated with impairment.

TABLE 1A

Functionally Grouped ACGs and Genes Showing Highly Significant Age-Dependent Decreases in Expression

GenBank	Description	Young	Mid	Age	ANOVA p	beh all
Synaptic Structural Plasticity						
M64780*	Agrn, Agrin	2746 ± 105	2334 ± 74	2207 ± 79	0.0005	Both
L21192	GAP-43, membrane attached signal protein 2 (brain)	10324 ± 546	8990 ± 327	8165 ± 480	0.0095	Both
S82649	Narp, neuronal activity-regulated pentraxin	4358 ± 300	3470 ± 143	3247 ± 185	0.0029	OMT
M74223	VGF, neurosecretory protein	6697 ± 373	5836 ± 387	4722 ± 369	0.0042	OMT
U63740*	Fez1, Protein kinase C-binding protein Zeta1	10339 ± 180	9322 ± 258	9388 ± 330	0.0239	OMT
AB003726	Homer1a, RuvB-like protein 1	3546 ± 270	2354 ± 121	2469 ± 132	0.0001	None
U19866	Arc, activity-regulated cytoskeleton-associated protein	6374 ± 527	4408 ± 228	4094 ± 398	0.0008	None
Transcription Regulator						
M18416	Egr1, Early growth response 1 (Krox-24)	4911 ± 259	3688 ± 177	3544 ± 165	0.0001	Both
M92433	NGFI-C, Zinc-finger transcription factor	2037 ± 149	1576 ± 44	1495 ± 70	0.0009	Both
L08595	Nuclear receptor subfamily 4, group A, member 2	1467 ± 80	1186 ± 83	1011 ± 62	0.0010	Both
AI030089	Nopp130, nucleolar phosphoprotein p130	471 ± 31	397 ± 31	314 ± 22	0.0022	Both
AF016387	RXRG, retinoid X-receptor gamma	1900 ± 129	1503 ± 95	1365 ± 103	0.0059	Both
AA800794	HT2A, zinc-finger protein	2480 ± 67	2396 ± 41	2097 ± 73	0.0004	OMT
AA799641	S164, Contains a PWI domain associated with RNA splicing	7645 ± 169	7690 ± 183	6842 ± 250	0.0106	OMT
U78102	Egr2, Early growth response 2	576 ± 95	223 ± 21	205 ± 23	0.0001	SWM
U44948	SmLIM, smooth muscle cell LIM protein	1166 ± 15	928 ± 55	887 ± 38	0.0001	SWM
AA891717	USF-1, upstream stimulatory factor 1	3607 ± 142	2993 ± 91	3025 ± 66	0.0003	None
AF095576	Aps, adaptor protein with pleckstrin and src homology	526 ± 40	275 ± 49	272 ± 46	0.0007	None
Intracellular Signal Transduction						
AI176689	MAPKK 6, mitogen-activated protein kinase kinase 6	2012 ± 84	1781 ± 92	1528 ± 88	0.0030	Both
X89703	TPCR19, Testis Polymerase Chain Reaction product 19	361 ± 25	320 ± 25	252 ± 24	0.0155	Both
L04485	MAPPK1, mitogen-activated protein kinase kinase 1	13110 ± 365	11951 ± 312	11200 ± 506	0.0104	OMT
AA817892	Gnb2, Guanine nucleotide binding protein (beta 2 subunit)	6500 ± 159	5606 ± 214	5765 ± 218	0.0110	OMT
AF000901	P58/P45, Nucleoporin p58	597 ± 43	444 ± 51	391 ± 47	0.0150	OMT
M87854	Beta-ARK-1, beta adrenergic receptor kinase 1	1994 ± 110	1723 ± 90	1544 ± 114	0.0202	OMT
AF058795	Gb2, GABA-B receptor	9443 ± 360	9064 ± 478	7857 ± 323	0.0228	OMT
AA800517	VAP1, vesicle associated protein	637 ± 72	674 ± 61	455 ± 35	0.0228	OMT
Signal Transduction						
AF003904	CRH-binding protein	773 ± 51	782 ± 35	630 ± 23	0.0119	Both
M15191	Tac1, Tachykinin	1415 ± 110	1078 ± 57	1068 ± 74	0.0093	OMT
AF091563	Olfactory receptor	440 ± 21	367 ± 29	332 ± 27	0.0233	SWM
M64376	Olfactory protein	810 ± 26	605 ± 83	568 ± 57	0.0247	SWM
M15880	Npy, Neuropeptide Y	4647 ± 158	3561 ± 223	3668 ± 141	0.0004	None
Adhesion, Extracellular Matrix						
M27207	Col1a1, Procollagen- type I (alpha 1)	678 ± 24	521 ± 43	480 ± 23	0.0005	Both
AF104362	Omd, Osteomodulin (osteoadherin)	289 ± 16	217 ± 24	185 ± 15	0.0024	Both
D63886	MMP16, matrix metalloproteinase 16	664 ± 23	604 ± 37	542 ± 19	0.0180	Both
M21354	Col3a1, collagen type III alpha-1	203 ± 22	157 ± 13	132 ± 9	0.0120	SWM
AB010437	CDH8, Cadherin-8	163 ± 24	100 ± 12	83 ± 17	0.0128	SWM

TABLE 1A
Functionally Grouped ACGs and Genes Showing
Highly Significant Age-Dependent Decreases in Expression

GenBank	Description	Young	Mid	Aged	ANOVA p	beh all
Metabolism						
L03294	Lpl, lipoprotein lipase	1147 ± 69	918 ± 40	749 ± 37	0.0000	Both
S68245	Ca4, carbonic anhydrase 4	2272 ± 75	1993 ± 63	1825 ± 54	0.0002	Both
AA859975	LOC64201, 2-oxoglutarate carrier	4792 ± 68	4370 ± 102	4255 ± 97	0.0010	Both
M24542	RISP, Rieske iron-sulfur protein	10337 ± 308	9095 ± 327	8833 ± 128	0.0013	Both
M18467	Got2, glutamate oxaloacetate transaminase 2	9470 ± 241	8355 ± 179	8332 ± 322	0.0061	Both
X64401	Cyp3a3, Cytochrome P450- subfamily IIIA (polypeptide 3)	805 ± 64	762 ± 51	581 ± 34	0.0089	Both
U83880	glycerol-3-phosphate dehydrogenase, mitochondrial	2054 ± 73	1988 ± 77	1673 ± 111	0.0127	Both
J05499	GLS, glutaminase (mitochondrial)	915 ± 24	844 ± 44	787 ± 14	0.0238	Both
U90887	Arg2, arginase type II	499 ± 21	374 ± 31	364 ± 22	0.0015	OMT
M22756	Ndufv2, mitochondrial NADH dehydrogenase (24 kDa)	12293 ± 574	10193 ± 670	9260 ± 750	0.0134	SWM
Transporters, Carriers						
L46873	Slc15a1, Oligopeptide transporter	426 ± 30	411 ± 24	292 ± 27	0.0028	Both
AB000280	PHT1, peptide/histidine transporter	802 ± 20	659 ± 40	691 ± 37	0.0198	OMT
U87627	MCT3, putative monocarboxylate transporter	687 ± 33	521 ± 22	480 ± 38	0.0002	SWM
AA799389	Rab3B, ras-related protein	353 ± 21	324 ± 25	251 ± 23	0.0150	SWM
Growth, Biosynthesis, Maintenance						
X16554	Prps1, Phosphoribosyl pyrophosphate synthetase 1	3159 ± 81	2747 ± 74	2637 ± 97	0.0006	Both
U66470	rCGR11, Cell growth regulator	820 ± 31	676 ± 31	662 ± 38	0.0051	Both
M37584	H2AZ, H2A histone family (member Z)	5335 ± 73	4906 ± 186	4600 ± 162	0.0090	Both
U90610	Cxcr4, CXC chemokine receptor	811 ± 56	812 ± 59	614 ± 29	0.0109	Both
AA874794	Bex3, brain expressed X-linked 3	16735 ± 376	14986 ± 588	14238 ± 457	0.0047	OMT
AA892506	coronin, actin binding protein 1A	4101 ± 121	3625 ± 114	3558 ± 135	0.0104	OMT
AA893939*	DSS1, deleted in split hand/ split foot protein 1	4201 ± 76	3860 ± 129	3658 ± 141	0.0149	OMT
AF087037	Btg3, B-cell translocation gene 3	652 ± 55	676 ± 71	460 ± 29	0.0163	OMT
U06099	Prdx2, Peroxiredoxin 2	12667 ± 675	11742 ± 641	10339 ± 272	0.0216	OMT
AI172476	Tieg-1, TGF-beta-inducible early growth response protein 1	1127 ± 99	925 ± 63	812 ± 53	0.0177	SWM
AA866411	Necdin, neuronal growth suppressor	1994 ± 81	1568 ± 86	1542 ± 62	0.0005	None
Protein Processing and Trafficking						
X54793	Hsp60, heat shock protein 60	10088 ± 333	9602 ± 299	8693 ± 229	0.0071	Both
AA875047	TCPZ, T-complex protein 1 (zeta subunit)	997 ± 161	728 ± 99	470 ± 59	0.0095	Both
D21799	Psmb2, Proteasome subunit (beta type 2)	7298 ± 242	6892 ± 229	6395 ± 177	0.0241	Both
U53922	Hsj2, DnaJ-like protein (RDJ1)	10716 ± 382	8836 ± 190	8392 ± 204	0.0000	SWM
X78605	rab4b, ras-homologous GTPase	3131 ± 292	2040 ± 196	2006 ± 135	0.0012	None

[42] For TABLE 1A, "GenBank" is the gene accession number established at the web accessible GenBank database <http://www.ncbi.nlm.nih.gov/>, The "Description" includes a 'common name' (if applicable) as well as a brief description of the gene product. Values for Young, Mid-Aged, and Aged categories are the mean ± SEM of expression values. Genes are put into functional categories (*see*, above) and grouped by their level of association with behavior (expression correlated significantly (Pearson's; $p \leq .025$) with both tasks, with the OMT, with the SWM, or with none of the tasks but highly significant across age ($p \leq .001$ on ANOVA across age, $p > .025$ for correlation on both SWM and OMT). Within each level of

association, genes are ranked by the significance of the age-dependent change in their expression level (ANOVA; $p \leq .025$). Asterisked (*) genes are those that also showed a significant behavioral correlation (Pearson; $p \leq .025$).

[43] ACGs that were downregulated with aging (TABLE 1A) appeared primarily to represent metabolic and neuronal functions (FIG. 3a).

[44] *Metabolism.* Multiple genes related to functions of the mitochondrial electron transport chain (*e.g.*, glycerol 3-phosphate dehdrogenase, NADH dehydrogenase, Rieske's iron-sulpher protein) were downregulated with aging (TABLE 1A). Moreover, we found aging-dependent downregulation of several genes related to pathways important for glucogenic amino acid catabolism, including glutaminase and arginase (TABLE 1A).

[45] *Synaptic Structural Plasticity.* One of the most prominent categories of identified genes showing decreased expression and behavioral correlation was that comprising genes involved in synaptic structural plasticity, including neurite outgrowth and synaptogenesis (*e.g.*, decreased expression of genes encoding agrin, GAP-43, Homer 1a, Narp, Arc, *etc.*) (TABLE 1A). Many of these genes are activity-dependent in neurons and have been linked previously to synaptic plasticity, neurite remodeling or learning in univariate studies (*e.g.*, Biewenga JE *et al., Acta Biochim Pol 43:* 327-38 (1996); Steward O *et al., Neuron 21:* 741-51 (1998), Mantych KB & Ferreira A, *J Neurosci 21:* 6802-9 (2001), Guzowski JF *et al., J Neurosci 20:* 3993-4001 (2000), Bezakova G, *et al. Proc Natl Acad Sci U S A 98* 9924-9 (2001)), although Gap-43 is one of the few reported so far to change with aging. Similarly, many other neural activity-dependent genes, including IEGs in the Transcription Regulators and Signaling categories (*e.g.*, Egr1, Egr 2, MAPKK, *etc.*), showed decreased expression with aging and were correlated with impaired cognition (TABLE 1A).

[46] In addition, multiple genes important for general growth and biosynthetic mechanisms, chaperone functions and protein processing were also downregulated with aging (*e.g.*, hsp60, histone H2AZ, proteasome subunit, DNA J-like homolog, *etc.*) as were specific neuronal signaling genes (*e.g.*, GluR 5-2, the kainate receptor; and neuropeptide Y) (TABLE 1A). These widely downregulated biosynthetic and signaling genes appear to reflect a general involution of metabolic and neurite structural remodeling processes in neurons (*e.g.*, FIG. 4, TABLE 1A). Chaperone proteins such as the DNA-J-like homolog and hsp60 play critical roles in preventing protein aggregates (Satyal SH *et al., Proc Natl Acad Sci U S A 97* 5750-5 (2000)), which are known to be critical in Alzheimer's disease (Price DL & Sisodia SS, *Annu*

Rev Neurosci 21: 479-505 (1998), Kovacs DM &Tanzi RE, *Cell Mol Life Sci 54:* 902-9 (1998); Sisodia SS *et al., Am J Hum Genet 65:* 7-12 (1999), Tanzi RE & Parson AB (2000), Selkoe DJ, *Neuron 32:* 177-80 (2001)), and could therefore have implications for age-dependent vulnerability to Alzheimer's disease.

TABLE 1B

ACGs and Genes Showing Highly Significant Age-Dependent Increases in Expression

GenBank	Description	Young	Mid	Aged	ANOVA P	beh all
Inflammation, Defense, Immunity						
J04488	Ptgds, Prostaglandin D synthase	3976 ± 248	6891 ± 350	8365 ± 438	0.0000	Both
X71127	c1qb, complement component 1- q (beta polypeptide)	885 ± 52	1461 ± 85	1895 ± 102	0.0000	Both
J03752	Microsomal GST-1, glutathione S-transferase	368 ± 43	695 ± 60	910 ± 45	0.0000	Both
L40362*	MHC class I RT1.C-type protein	1755 ± 64	2106 ± 82	2501 ± 77	0.0000	Both
U17919	Aif1, allograft inflammatory factor 1	712 ± 29	990 ± 47	1152 ± 67	0.0000	Both
M15562	MHC class II RT1.u-D-alpha chain	608 ± 73	1194 ± 238	2120 ± 173	0.0000	Both
X13044	Cd74, CD74 antigen	-49 ± 44	155 ± 83	603 ± 100	0.0000	Both
M24324	RTS, MHC class I RT1 (RTS) (u haplotype)	3274 ± 175	4599 ± 363	5822 ± 342	0.0000	Both
M32062	Fcgr3, Fc IgG receptor III (low affinity)	347 ± 25	462 ± 32	557 ± 21	0.0000	Both
AJ222813	Il18, interleukin 18	110 ± 33	208 ± 14	261 ± 16	0.0002	Both
L40364	RT1Aw2, RT1 class Ib	2033 ± 126	2546 ± 127	2842 ± 115	0.0004	Both
AI231213	Kangai 1, suppression of tumorigenicity 6	2727 ± 116	2952 ± 120	3484 ± 139	0.0008	Both
AI170268	Ptgfr, Prostaglandin F receptor	6651 ± 248	8057 ± 336	8502 ± 359	0.0013	Both
X52477	C3, Complement component 3	34 ± 49	236 ± 83	476 ± 100	0.0034	Both
X73371	FCGR2, Low affinity immunoglobulin gamma Fc receptor II	218 ± 19	285 ± 24	384 ± 21	0.0001	OMT
X78848	Gsta1, Glutathione-S-transferase (alpha type)	3145 ± 74	3909 ± 188	4155 ± 204	0.0009	OMT
AA818025*	Cd59, CD59 antigen	6465 ± 265	7269 ± 163	7474 ± 189	0.0052	OMT
AA891810	GST, Glutathione S-transferase	1136 ± 83	1411 ± 70	1791 ± 101	0.0001	SWM
U92081	Gp38, Glycoprotein 38	547 ± 26	679 ± 38	802 ± 66	0.0037	SWM
X62322	Grn, Granulin	4514 ± 145	4972 ± 254	5375 ± 119	0.0116	SWM
Transcription Regulator						
X13167*	NF1-A, nuclear factor 1 A	112 ± 30	265 ± 38	300 ± 26	0.0008	Both
U67082	KZF-1, Kruppel associated box (KRAB) zinc finger 1	472 ± 31	565 ± 32	617 ± 29	0.0099	Both
U92564	Roaz, Olf-1/EBF associated Zn finger protein	429 ± 50	687 ± 71	761 ± 50	0.0014	OMT
L16995	ADD1, adipocyte determ./ different.-dependent factor 1	784 ± 100	1054 ± 75	1179 ± 95	0.0160	OMT
AI237535	LitaF, LPS-induced TNF-alpha factor	979 ± 62	1078 ± 68	1338 ± 114	0.0193	OMT
AI177161	Nfe2l2, NF-E2-related factor 2	544 ± 31	590 ± 36	687 ± 25	0.0096	SWM

TABLE 1B

ACGs and Genes Showing Highly Significant Age-Dependent Increases in Expression

GenBank	Description	Young	Mid	Aged	ANOVA P	beh all
Signal Transduction						
U26356	S100A1, S100 protein (alpha chain)	1382 ± 105	1636 ± 76	1999 ± 115	0.0008	Both
AA850219	Anx3, Annexin A3	438 ± 26	501 ± 21	575 ± 26	0.0023	Both
D84477	Rhoa, ras-related homolog A2	749 ± 108	1069 ± 111	1319 ± 85	0.0024	Both
AF048828	VDAC1, voltage-dependent anion channel 1	2334 ± 294	3157 ± 392	3844 ± 290	0.0137	Both
AI102103	Pik4cb, Phosphatidylinositol 4-kinase	975 ± 63	1029 ± 67	1252 ± 80	0.0247	Both
L35921	Ggamma, GTP-binding protein (gamma subunit)	498 ± 30	543 ± 43	712 ± 64	0.0108	SWM
M83561	GluR-5, kainate sensitive glutamate receptor	248 ± 23	359 ± 22	351 ± 12	0.0007	None
Adhesion, Extracellular Matrix						
E13541	Cspg5, chondroitin sulfate proteoglycan 5	3938 ± 342	5112 ± 312	5980 ± 242	0.0003	Both
X83231	PAIHC3, Pre-alpha-inhibitor, heavy chain 3	2586 ± 110	2974 ± 180	3460 ± 183	0.0038	OMT
AF097593	Ca4, cadherin 2- type 1 (neuronal)	615 ± 45	855 ± 61	881 ± 59	0.0049	OMT
Myelin-Related Proteins						
M55534	Cryab, alpha crystallin polypeptide 2	2889 ± 155	4153 ± 196	4621 ± 238	0.0000	Both
D28111	MOBP, myelin-associated oligodendrocytic basic protein	13950 ± 386	15483 ± 633	18407 ± 909	0.0004	Both
X06554	S-MAG, myelin-associated glycoprotein C-term	5282 ± 258	5595 ± 140	6564 ± 326	0.0038	Both
S55427	Pmp, peripheral myelin protein	2458 ± 59	2856 ± 148	3080 ± 129	0.0051	OMT
M22357	MAG, myelin-associated glycoprotein	978 ± 163	1544 ± 190	2455 ± 332	0.0010	SWM
Lipid Metabolism/ Transport						
X54096	Lcat, Lecithin-cholesterol acyltransferase	187 ± 35	298 ± 30	417 ± 38	0.0003	Both
S83279	HSDIV, 17-beta-hydroxysteroid dehydrogenase type IV	630 ± 54	685 ± 91	928 ± 67	0.0182	Both
U37138	Sts, Steroid sulfatase	368 ± 74	521 ± 33	587 ± 35	0.0128	OMT
X55572	Apod, Apolipoprotein D	5875 ± 355	7281 ± 601	8343 ± 595	0.0133	OMT
L07736	Cpt1a, Carnitine palmitoyltransferase 1 alpha (liver)	599 ± 65	677 ± 59	854 ± 59	0.0192	OMT
Amino Acid/ Transmitter Metabolism						
J03481	DHPR, Dihydropteridine reductase	13260 ± 369	16897 ± 528	17432 ± 380	0.0000	Both
Z50144	Kat2, kynurenine aminotransferase II	106 ± 33	183 ± 19	240 ± 24	0.0040	Both
U07971	Transamidinase, mitochondrial	2897 ± 130	3311 ± 186	3644 ± 182	0.0183	OMT
M77694	Fah, fumarylacetoacetate hydrolase	847 ± 36	990 ± 49	1305 ± 98	0.0002	SWM
Cytoskeletal, Vesicle Fusion						
X62952	Vim, vimentin	571 ± 100	998 ± 162	1346 ± 122	0.0016	Both
AA892333	Tuba1, alpha-tubulin	-52 ± 83	117 ± 90	357 ± 79	0.0080	Both
U11760*	Vcp, valosin-containing protein	4314 ± 234	5004 ± 333	5651 ± 278	0.0120	Both
U32498*	RSEC8, rat homolog of yeast sec8	-11 ± 37	270 ± 81	232 ± 82	0.0236	OMT
AF083269*	P41-Arc, actin-related protein complex 1b	406 ± 23	488 ± 49	626 ± 72	0.0249	OMT
AF028784	GFAP, glial fibrillary acidic protein	19860 ± 714	19731 ± 1002	23241 ± 1058	0.0217	SWM
Transporters, Carriers						
M94918	Hbb, beta hemoglobin	6172 ± 737	8698 ± 646	13715 ± 1017	0.0000	Both
U31866	Nclone10	3625 ± 302	5416 ± 561	7407 ± 511	0.0000	Both
D38380	Tf, Transferrin	11990 ± 728	16431 ± 707	19831 ± 1519	0.0001	Both
X56325	Hba1, alpha 1 hemoglobin	14433 ± 611	17259 ± 959	23893 ± 1426	0.0000	OMT
AF008439	Natural resistance-associated macrophage protein 2	69 ± 17	153 ± 19	152 ± 13	0.0018	SWM

TABLE 1B

ACGs and Genes Showing Highly Significant Age-Dependent Increases in Expression

GenBank	Description	Young	Mid	Aged	ANOVA P	beh all
Growth, Biosynthesis, Maintenance						
AA799645	FXYD domain-containing ion transport regulator 1	1680 ± 58	2025 ± 68	2457 ± 129	0.0000	Both
L03201	Ctss, cathepsin S	17087 ± 393	19066 ± 691	22376 ± 875	0.0001	Both
M27905	Rpl21, Ribosomal protein L21	11279 ± 905	13999 ± 389	15557 ± 379	0.0001	Both
AA893493	RPL26, Ribosomal protein L26	18442 ± 688	23043 ± 506	24252 ± 1162	0.0001	Both
X52619	Rpl28, Ribosomal protein L28	13167 ± 323	13231 ± 310	14520 ± 228	0.0034	Both
X14181*	RPL18A, Ribosomal protein L18a	8623 ± 430	10171 ± 389	11025 ± 602	0.0068	Both
M31076	TNF-alpha, Transforming growth factor (alpha)	139 ± 23	241 ± 43	295 ± 35	0.0167	Both
AI171462*	Cd24, CD24 antigen	864 ± 69	1270 ± 86	1304 ± 101	0.0026	OMT
X68283	Rpl29, Ribosomal protein L29	9705 ± 262	9500 ± 300	10807 ± 267	0.0050	OMT
X53504*	RPL12, Ribosomal protein L12	9877 ± 328	11398 ± 367	11719 ± 620	0.0241	OMT
U77829	Gas-5, growth arrest homolog	173 ± 15	228 ± 14	264 ± 20	0.0030	SWM
AI234146	Csrp1, Cysteine rich protein 1	4436 ± 335	4925 ± 207	5451 ± 179	0.0243	SWM
Protein Processing and Trafficking						
M32016	Lamp2, lysosomal-associated membrane protein 2	759 ± 38	906 ± 36	1092 ± 74	0.0008	Both
E01534	Rps15, Ribosomal protein S15	16577 ± 368	17202 ± 429	18363 ± 368	0.0116	OMT
AI028975	AP-1, adaptor protein complex (beta 1)	1077 ± 38	1163 ± 69	1317 ± 49	0.0158	OMT
AI175486	Rps7, Ribosomal protein S7	5820 ± 448	6409 ± 312	7212 ± 208	0.0215	OMT
AF023621	Sort1, sortilin	414 ± 34	813 ± 143	812 ± 109	0.0247	OMT
AI230712	Pace4, Subtilisin - like endoprotease	281 ± 31	447 ± 49	570 ± 56	0.0010	SWM
AA891445*	Skd3, suppressor of K^+ transport defect 3	321 ± 24	440 ± 42	508 ± 37	0.0043	SWM
AF031430	Stx7, Syntaxin 7	794 ± 133	1387 ± 188	1461 ± 122	0.0097	SWM
AA900516	Pdi2, peptidyl arginine deiminase (type II)	57 ± 42	314 ± 62	344 ± 51	0.0015	None

[47] The analyses for TABLE 1B are as described for TABLE 1A.

[48] *Upregulated Genes.* Genes that were upregulated with aging and negatively correlated with behavior fit primarily into categories that appeared to reflect activated glial functions (FIGS. 3B and 5, TABLE 1B). Additionally, among the main unexpected findings was a widespread upregulation in the expression of genes encoding proteins for myelin synthesis and lipid turnover (TABLE 1B).

[49] *Lipid Metabolism.* Multiple genes important for mitochondrial and cytosolic lipid β-oxidation (*e.g.*, carnitine palmitoyltransferase, lecithin-cholesterol acyltransferase, *etc.*; TABLE 1B), the primary pathway for free fatty acid (FFA) catabolism, were upregulated.

[50] *Increased Myelin Synthesis, Cholesterol Biogenesis and Vesicle Transport.* Importantly for identifying the trigger mechanism for elevated lipid catabolism, the expression of many genes encoding myelin-related proteins or myelin-related transcription factors on the microarray was increased with aging (and several also were correlated with cognitive impairment) (TABLE 1B). These observations strongly suggest that a major increase in myelin synthesis programs developed with aging. This interpretation is also supported by the upregulation of multiple genes important in lipogenesis for cholesterol

biosynthesis (Add 1/SREBP1), and the packaging/transport of cholesterol esters and other complex lipids (ApoD, LCAT, *etc.*) (TABLE 1B). Recent studies have shown that stimulation of myelin synthesis programs in oligodendrocytes is associated with induction of genes for both myelin proteins and lipogenic pathways (Nagarajan R *et al., Neuron 30* 355-68 (2001)).

[51] *Cyloskeleton/Vesicles*. Moreover, expression of genes related to actin assembly, transport or fusion of packaged vesicles (actin related complex, rsec8, tubulin, and syntaxin 7) was increased (TABLE 1B). These molecules are associated with vesicle transport and fusion in neurons. In addition, however actin assembly proteins are also known to play a major role in myelin vesicle transport in oligodendrocytes (Madison DL *et al., J Neurochem 72:* 988-98 (1999)). Given the upregulation of myelin programs and the downregulation of synaptic plasticity genes, therefore, the age-dependent upregulation of genes linked to vesicle transport capacity seems more likely to be associated with enhanced myelin transport in oligodendrocytes. Further support for the view that extensive oligodendrocyte activation and/or synthesis occurs in hippocampal aging is provided by the observation that many genes that were upregulated with aging are preferentially expressed in oligodendrocytes (*e.g.*, myelin proteins, FAH, PGD-S, *etc.*) (*e.g.*, Labelle Y *et al., Biochim Biophys Acta 1180:* 250-6 (1993)).

[52] Myelin also is normally degraded to free fatty acids through the endosomal-lysosomal pathway. Consistent with elevation of myelin degradation, we also found increased expression of Cathepsin S and other genes encoding lysosomal enzymes (TABLE 1B). Cathepsin S is particularly important in the processing of antigenic myelin fragments.

[53] *Amino Acids*. In contrast to enzymes for glucogenic amino acids (TABLE 1A), expression was upregulated for multiple genes encoding enzymes related to the metabolism of the ketogenic/glucogenic amino acids, tyrosine, phenylalanine and tryptophan (*e.g.*, DHPR, KAT, FAH, *see,* TABLE 1B). Catabolism of ketogenic amino acids yields either acetoacetate or one of its precursors (*e.g.*, acetyl CoA), which can be used either for energy metabolism or lipogenesis. Upregulation of DHPR, which catalyzes the formation of a critical cofactor (tetrahydrobiopterin) for tyrosine and monoamine synthesis, and concomitant upregulation of MAO-B (TABLE 3), together suggest elevated metabolism of tyrosine and tryptophan via greater monoamine turnover.

[54] *Inflammation/Defense/Immunity.* There was massive upregulation of expression of genes encoding MHC class I antigen presenting molecules, and numerous other inflammatory/immune proteins (TABLE 1B). Genes in the inflammation category exhibited some of the most robust monotonic changes with aging seen in our results using the method of the invention (*e.g.*, most were significant at the $p<0.001$ criterion with 0.025 FDR) (TABLE 1B). Moreover, most were inversely correlated with cognitive function (FIG. 3B).

[55] Consistent with evidence of a role for oxidative stress in brain aging (Carney JM *et al., Proc Natl Acad Sci U S A 88:* 3633-6 (1991), Hensley K *et al., Ann N Y Acad Sci 786:* 120-34 (1996), Bickford PC *et al., Brain Res 866:* 211-7 (2000), Lee CK, *et al. Nat Genet 25:* 294-7 (2000), Jiang CH *et al., Proc Natl Acad Sci U S A 98:* 1930-4 (2001)), we also found increased expression for molecules important in defense against oxidative stress (GST, GSTa1) (TABLE 1B). One potentially key new finding here, as noted above, was that DHPR was upregulated with aging and correlated with cognitive decline (TABLE 1B). Its product, tetrahydrobiopterin, is also an essential cofactor for nitric oxide synthase (Boyhan A, *et al. Biochem J 323 (Pt 1)* 131-9 (1997)). Because oxyradicals formed from nitric oxide appear to play a major role in inflammatory neuronal damage (Bal-Price A & Brown GC, *J Neurosci 21:* 6480-91 (2001), Calingasan NY & Gibson GE, *Brain Res 885:* 62-9 (2000)), this may be an important pathway through which the deleterious effects of inflammation are mediated in brain aging.

[56] *Glial Markers.* Astrocyte reactivity and astrocyte markers are also well recognized to increase in the aged rodent and human hippocampus (Landfield PW *et al., Science 214:* 581-4 (1981), Landfield PW *et al., J Neurobiol 23:* 1247-60 (1992), Nichols NR *et al., Neurobiol Aging 14:* 421-9 (1993), Finch CE & Longo VD, *Neuroinflammatory Mechanisms in Alzheimer's Disease: Basic and Clinical Research,* 237-256 (2001)) and the present data confirm extensive upregulation of genes (Finch CE & Tanzi RE *Science 278:* 407-11 (1997)) for glial markers (*e.g.*, vimentin, GFAP - cytoskeleton category, TABLE 1B). In addition, we extended those observations to show that genes for proteoglycans (TABLE 1B) and other extracellular proteins (*e.g.*, fibronectin) that are components of astroglial scars also were upregulated. These changes may reflect astroglial-mediated reorganization of the extracellular matrix, a process known to be unfavorable for axonal remodeling.

[57] *Signal Transduction.* Several genes in calcium regulating and G-protein-coupled signaling pathways were also identified (TABLE 1B). In particular, S100A1, which

modulated Ca^{2+}-induced Ca^{2+} release, and PI 4-kinase, which acts to produce IP3 were upregulated. Several other S100-related genes (*e.g.*, S100A4 and P9K2; TABLE 3) were also upregulated with aging but failed to meet the strict criteria set forth herein (FIG. 2).

[58] *Biosynthesis*. Concomitantly, many ribosomal (growth) and protein processing genes were upregulated (TABLE 1B). The upregulated changes reflect increased protein synthesis, turnover and phagocytosis associated with strongly elevated biosynthetic processes in glial compartments (*e.g.*, elevated myelin, MHC, proteoglycan synthesis).

[59] *Orchestrating Factors*. Our data show that a number of transcriptional regulators and cytokines, including KZF-1, Roaz and members of the NFI family (TABLE 1B) were upregulated and therefore, may be strong candidates for coordinating factors. Under some conditions, several of these factors function as negative transcriptional regulators.

[60] *Relationship to Fold Change*. The large majority of microarray analyses to date have used fold-change criteria to detect changes in expression. In addition to providing little basis for statistical assessment (*e.g.*, Miller RA, *et al. J Gerontol A Biol Sci Med Sci 56* B52-7 (2001)), however, fold-change criteria are relative insensitive. Among the 139 ACGs, most exhibited group mean fold changes between the Young and Aged groups of less than 1.5 (92), a few showed fold changes between 1.5 and 2.0 (26), and only a handful of genes exceeded 2-fold-change (20) (TABLES 1A and B). Thus, few of our results using the method of the invention would have been detected in the great majority of prior microarray studies, in which 1.7 to 2-fold change cutoffs are commonly used as minimum criteria for identifying differences, and many changes are reported in the 3 - 4 fold range. Further, the rank order correlation between group mean fold-change and p values on the ANOVA for all aging-significant genes, although significant, was modest according to Spearman's correlation test (Spearman's $r = 0.45$, $p < 0.001$). Armitage P & Berry G, *Statistical Methods in Medical Research, 2nd Edn.*, 200-205 (1987). This indicates that fold-change accounted for only ~ 20% of the variance (r2) in the degree of statistical significance on the ANOVA. Some of our results detected with the enhanced sensitivity of statistical analysis were extremely subtle (*e.g.*, 1.1 fold for the L28 and L29 ribosomal proteins, TABLE 1B). Despite this enhanced sensitivity, however, numerous false negatives were still undoubtedly present in our data set.

[61] *Age Course of Gene Expression Changes*. Using a design with three age groups enabled us to classify genes and categories according to their general patterns of age dependence of change (FIGS. 4 and 5). Genes were classified by whether 75% of the

maximal change occurred between the Young and Mid-Aged groups (Yng to Mid), the Mid-Aged and Aged groups (Mid to Aged), or the Young and Aged groups (monotonic).

[62] Almost all categories comprising downregulated and cognitively correlated genes (TABLE 1A), exhibited their greatest change between the Young and Mid-Aged points, and many did not show much additional downregulation between the Mid-Aged and Aged groups (FIG. 4). This was also true for the entire population of genes whose expression decreased with aging at $p < 0.025$ (pie-chart inset, FIG. 4). Conversely, by far the largest fraction of functional categories of upregulated genes showed a monotonic age course of change that also began between the Young and Mid-Aged points but, in addition, continued between the Mid-Aged and Aged points (FIG. 5). However, the Cytoskeletal and Transcriptional Regulator categories contained significant numbers of exceptions that exhibited >75% of their change between the Young and Mid-Aged groups (TABLE 1B). Additionally, among all genes that showed significant upregulation with aging, the majority fit the monotonic classification (pie-chart inset, FIG. 5). Only a few scattered genes showed a predominantly Mid to Aged change pattern (*e.g.*, FIGS. 4 and 5 pie-charts).

[63] *Strongest Correlations of Pathways with Memory Performance.* To determine which pathways were most closely correlated with memory performance, we calculated the percentage of genes in each of our categories that were correlated significantly (at $p < 0.025$) with both memory tests. We reasoned that each test measures aspects of memory but each test also has its own error sources and confounding contributions from non-cognitive performance factors. Therefore, genes that correlated with both tasks seem more likely to be associated with cognitive processes.

[64] Because memory performance changed most between the Mid-Aged and Aged groups (FIG. 1), whereas downregulated genes changed little (FIG. 4) and upregulated genes continued to increase (FIG. 5) between those groups, the pattern of age course changes relative to cognitive performance was more similar for upregulated than for downregulated genes. Not surprisingly, therefore, more upregulated (52%) than downregulated (44%) genes were correlated with performance on both tasks. Three categories of downregulated genes had 50% or higher both-task correlations: Adhesion and extracellular matrix (3/5), Metabolism (8/10), and Protein processing and trafficking (3/5). Whereas seven categories of upregulated genes had 50% or higher both-task correlations: Signaling (5/7), Inflammation (14/20), Cytoskeleton/Vesicle (3/6), Myelin related proteins (3/5), Amino acid/ transmitter

metabolism (2/4), Transporters and carriers (3/5), and Growth, biosynthesis, maintenance (7/12). In the Signaling category, moreover, genes involved in intracellular Ca^{2+} release, S100A1 and PI3-K (TABLE 1B), were correlated with both tasks.

[65] Another way to examine closeness of correlation specifically with memory impairment is to correlate gene expression with performance only in the aged group. This correlation focuses on variation in the performance of aged animals and removes the overall age course pattern from contributing to the correlation with impairment. This correlation is independent of the ANOVA for aging effects and an FDR also can be calculated. Consequently, we tested each of the 139 primary aging- and behaviorally-related genes for correlation with 24 hr memory performance on the OMT in the aged group. The OMT was selected over the SWM for this test as it had the greater dispersion of performance needed for correlation analysis. The correlation tests in the aged group ($n = 10$) of course had considerably less power than across all three groups ($n = 29$) and the criterion for significance was set at $p < 0.025$.

[66] Only 3 (4.9%) of the downregulated ACGs, but 10 (12.2%) of the upregulated ACGs were correlated with Aged group performance on the OMT. The FDR for these genes was 0.28. Two of the 3 downregulated ACGs were accounted for by the Synaptic Structural Plasticity category (Fez-1, agrin). For upregulated genes, two of the 10 ACGs were from Inflammation (MHC and CD59 antigen), three from Cytoskeleton/Vesicle category (Vcp, rsec8 and p41-Arc), and three from Growth/Biosynthesis (2 ribosomal proteins and CD24 antigen). No other category had more than one, including Transcription (NF1-A) and Protein processing and trafficking (Skd3).

[67] Thus, by the criterion of correlation on both tasks, the upregulated categories of Inflammation/Immune, signaling (particularly Ca^{2+} signaling), Cytoskeleton/Vesicle and Amino Acid Metabolism were ranked most highly. By the criterion of correlation in the aged group only, the upregulated categories of Cytoskeleton/Vesicle (3/6), Biosynthesis (3/12) and Inflammation (2/20), and the downregulated category of Synaptic Plasticity (2/7) were ranked most highly.

[68] *Benefits of the Invention*. One of the major problems associated with developing treatments for aging-dependent functional decline is the lack of good genomic biomarkers or targets of brain aging needed for evaluating the efficacy of different treatments. Our ACGs, therefore, could serve as excellent biomarkers of cognitive aging. Using microarrays

constructed to contain oligonucleotide sequences specific for hybridization with and measurement of mRNAs of the identified ACGs, laboratory animals could be assessed for degree of cognitive aging before, during and after treatment with a compound. Treatments that slowed or reversed the ACG profile during aging might be highly promising for development as new therapeutic approaches. Further, treatments that slowed or reversed expression profiles of particular genes in our panel of biomarkers might reveal which specific genes among the subset of ACGs are most critical for the age-dependent functional decline and, therefore, would suggest genes and gene products that should be targeted with high priority for development of therapeutic interventions. The same approach could be applied using our panel of unique brain aging genes that are not specifically clustered with cognition related genes, to evaluate and develop new therapies and compounds for treatment of brain aging in general.

[69] The panel of ACGs identified here can be used on a microarray to perform diagnostic tests. Subjects suspected of having accelerated brain aging or early age-related neurodegenerative disease could provide a small brain biopsy sample for testing by microarray. This could then determine the subject's suitability for pharmacologic intervention.

[70] Based on the gene lists described above, investigators can develop new drugs or treatments aimed at altering the activity of one or more genes in the lists, or products encoded by those genes, or targets of the products, with the goal of counteracting age-related cognitive impairment or brain aging in general.

[71] A smaller subset of ACGs, specifically linked to some process or system (*e.g.*, to inflammation, mitochondrial function, or lipid metabolism, *etc.*), could be used in a microarray to test efficacy of a new compound targeted to slowing or reversing aging and cognitive changes dependent on that set of genes or gene-impacted systems, either in experimental tests to develop new compounds, or as diagnostic or therapeutic guides.

[72] *Relevance to Human Brain Aging and Alzheimer's Disease.* Normal human brain aging is associated with memory dysfunction and appears to set the stage for Alzheimer's disease and other age-related neurodegenerative conditions. It also shares many features with animal models of aging. Landfield PW *et al.*, *J Neurobiol 23*: 1247-1260 (1992). Thus, many of the memory-correlated gene expression profiles seen here in rats may have implications for genomic mechanisms of human brain aging and/or Alzheimer's disease. This view is

supported by several parallels between processes identified here and those seen in human aging or Alzheimer's disease. For example, myelin abnormalities are also found extensively in normal brain aging in humans (leukoaraiosis). These white matter changes in humans are also correlated with cognitive dysfunction and become more severe in disease states. Further, cerebral metabolism begins to decline by mid-life in humans, much as it apparently does in rats (FIG. 4). Of particular note in light of our findings on oxidative phosphorylation and myelin turnover, mitochondrial diseases in humans also can result directly in demyelination.

[73] It is interesting, in view of the apparently altered lipid metabolism seen here, that activity of the cholesterol ester synthesizing enzyme acyl CoA:cholesterol acyltransferase (ACAT) is elevated in Alzheimer's disease and appears directly coupled to amyloid production. ACAT has lipogenic functions somewhat similar to those of LCAT, which was also upregulated here (TABLE 1A). Moreover, activity of glycerol-3-phosphate dehydrogenase (GPDH) is elevated in association with abnormal glucose metabolism in brains of patients with Down's syndrome. The gene encoding this glycolytic enzyme was also upregulated here (TABLE 1A). Other processes found in human aging or Alzheimer's disease brain for which we found corollaries in gene expression include, as noted, inflammation, oxidative stress and elevated KatII (kynurenine aminotransferase 2), among others. Thus, if these parallels depend, at least in part, on similar mechanisms, our our results show that widespread genomic regulatory changes would reasonably be expected to contribute to altered cerebral metabolism, lipid synthesis, neural activity and myelination in human brain aging as well.

[74] *Implications for a New Hypothesis of Brain Aging.* Based on the functional implications of our results, as discussed above, we provide a new working model of brain aging (FIG. 6). Early in adult life (*i.e.*, before mid-life) a series of brain changes begin, perhaps initiated by new expression of genes that exert deleterious late-life actions (*e.g.*, "late genes") (Finch CE, *Longevity, Senescence and the Genome,* 37-42 (Univ. Chicago Press, Chicago, 1990); Austad, SN, *Why We Age: What Science Is Discovering about the Body's Journey Through Life* (Indianapolis, Wiley, 1999)) or by catabolic hormonal processes (*e.g.*, glucocorticoids, Porter NM & Landfield PW, *Nature Neurosci 1*: 3-4 (1998)). These changes include reduced neuronal activity and induce a subtle shift from anabolic to catabolic metabolism in neurons. In neurons, the reduced anabolic capacity leads to diminished capacity for protein biosynthesis and, in particular, for activity-dependent neurite remodeling

and synaptogenesis. Concomitantly, an increase in degradation of myelin and lipids begins, perhaps triggered by reduced neural activity, or reduced oxidative phosphorylation and/or demand for an alternative energy source, or by an immune process similar to multiple sclerosis, among other possibilities. The degenerating myelin fragments are endocytosed in microglia and astrocytes, degraded by lysosomes and packaged into antigen-presenting MHC molecules. This in turn activates orchestrating cytokines and transcription factors that trigger an inflammatory reaction in the glia and possibly, in macrophages. The inflammation further accelerates the phagocytosis and degradation of myelin. As astrocytes hypertrophy, they increase glycolytic metabolism and synthesize "glial scar" proteins (*e.g.*, fibronectins, proteoglycans) that alter the extracellular matrix. In oligodendrocytes, lipogenic and myelin synthesis programs are activated in response to the ongoing demyelination and/or altered signaling pathways. In turn, remyelination may increase demand for lipid substrate and thereby also accelerate demyelination. Thus, positive feedback cycles between demyelination and myelination and/or between demyelination and inflammation, among other processes, might develop and further drive cellular dyshomeostasis. Eventually, the reduced synaptogenic capacity unfavorable extracellular matrix and degradative inflammatory processes result in failure of cognitive processing. Additionally, the ongoing catabolic processes erode neuronal membranes and cytoskeletons, increase protein aggregation and enhance vulnerability to neurodegenerative disease. Accordingly, our results, in conjunction with this working model, point directly to potentially useful therapeutic interventions and should, therefore, facilitate the design of such future therapeutics.

[75] The details of one or more embodiments of the invention are set forth in the accompanying description above. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. Other features, objects, and advantages of the invention will be apparent from the description and from the claims. In the specification and the appended claims, the singular forms include plural referents unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All patents and publications cited in this specification are incorporated by reference.

[76] The following EXAMPLES are presented in order to more fully illustrate the preferred embodiments of the invention. These examples should in no way be construed as limiting the scope of the invention, as defined by the appended claims.

EXAMPLE 1

BEHAVIORAL RESULTS

[77] Thirty animals in three age groups (n = 10/group) were trained sequentially on two tasks, first in the Morris spatial water maze (SWM) and then in the object memory task (OMT). Male Fischer 344 rats aged 4 months (Young, n = 10), 13 months (Mid-Aged, n = 10) and 24 months (Aged, n = 10) were used. Overall, the training/testing lasted seven days, and hippocampal tissue was collected 24 hr later. Training or testing occurred on each day except for the 2nd and 3rd days of the seven-day sequence.

[78] Methods used here for cognition assessment in the Morris Spatial Water Maze (SWM), a task sensitive to both hippocampal function and aging, have been described previously Norris CM & Foster TC, Neurobiol Learn Mem 71, 194-206 (1999). Briefly, rats were trained in a black tank, 1.7 M in diameter, filled with water (27±2° C). Behavioral data were acquired with a Columbus Instruments tracking system. After habituation to the pool, animals were given cue training with a visible platform (five blocks of three trials, maximum of 60 sec/trial, 20 sec intertrial interval and a 15 min interval between blocks). Rats remained in home cages under warm air after each block. Cue training was massed into a single day and the criterion for learning was finding the platform on 4 of the last 6 trials. For all animals that met this criterion, spatial discrimination training was initiated three days later in which the escape platform was hidden beneath the water but remained in the same location relative to the distal cues in the room. Fifteen min following the end of spatial training, a 1-min duration free-swim probe trial with the platform absent was administered, during which crossings over the former platform site (platform crossings) were recorded to test acquisition, followed by a refresher training block. Retention for platform location was again tested 24-hr later using a second 1-min free-swim probe trial.

[79] During cue training in the SWM, all animals were able to locate the visible escape platform according to our criteria and therefore, were trained on the hidden platform spatial task. During acquisition, Aged animals performed more poorly (longer latencies) than Mid-Aged or Young. In addition, an aging-dependent decrease in 24 hr retention, measured

by platform crossings (1-way ANOVA, $p < 0.05$), was observed on the retention probe trial (FIG. 1A). *Post hoc* analysis indicated that Young and Mid-Aged animals exhibited more platform crossings relative to Aged animals, but did not differ from each other.

[80] Methods used here for cognition assessment in the object memory task OMT) have been described previously by Ennaceur A & Delacour J, *Behav Brain Res 31*: 47-59. (1988). The object memory task (OMT) is also both sensitive to hippocampal function and affected by aging but is less dependent on physical strength and endurance. On the afternoon of the final spatial maze probe trial, animals were administered a habituation session (15-min) in the empty mesh cage to be used for the OMT (63.5 cm X 63.5 cm). OMT training began 24 hr after habituation and consisted of a 15-min acquisition session during which two 3-dimensional objects were placed at opposite sides of the cage, followed by two 15-min retention test sessions at 1 and 24 hr posttraining. During the acquisition session, the cage contained two sample objects (A and B) and the time spent actively exploring each object was recorded. After 1 hr, the rat was reintroduced into the cage and the time spent exploring a novel object, C, relative to the familiar object, B, was recorded. On the 24 hr test, familiar object A was reintroduced and object B was replaced by a second novel object, D. Objects were randomized across individuals and timed measures of exploration were used to calculate a memory index (MI) as follows: MI = (N-F)/T, where *N* is time spent exploring the novel object, *F* is time spent exploring the familiar object, and *T* is total time spent exploring the two objects. More time spent exploring the novel object (higher MI) is considered to reflect greater memory retention for the familiar object.

[81] In the OMT, Aged animals performed as well as Young or Mid-Aged on the 1 hr retention test (not shown), but there was a significant age-related decline in recall (1-way ANOVA, $p<.001$, for the main effect of age) on the 24 hr test (FIG. 1B). At 24 hr, Young and Mid-Aged groups were significantly different from the Aged group, but not from one another (Young vs. Aged: $p<.001$; Mid-Aged vs. Aged: $p<.05$; Young vs. Mid-Aged: N.S., Tukey's *post-hoc* test; Armitage P & Berry G, *Statistical Methods in Medical Research, 2nd Edn.*, 200-205 (1987)).

EXAMPLE 2

GENE MICROARRAY CHIP RESULTS

[82] Microarray analyses were performed on hippocampal CA1 tissues from each of the same behaviorally characterized 30 animals (one chip per animal), but one chip was lost for technical reasons, leaving a data set of 29 microarrays (Young = 9, Mid-Aged = 10, Aged = 10). For tissue preparation, twenty-four hours after completion of the OMT testing, animals were anesthetized with CO_2 gas and decapitated. The brains were rapidly removed and immersed in ice-cold, oxygenated artificial cerebrospinal fluid consisting of (in mM): 124 NaCl, 2 KCl, 1.25 KH_2PO_4, 2 $MgSO_4$, 0.5 $CaCl_2$, 26 $NaHCO_3$, and 10 dextrose. Hippocampi were removed and the CA1 region from one hippocampus per animal were dissected by hand under a stereomicroscope. The CA1 tissue block from each animal was placed in a microcentrifuge tube and flash frozen in dry ice for RNA isolation.

[83] For RNA isolation, total RNA was isolated using the TRIzol reagent and following the manufacturer's RNA isolation protocol (Gibco BRL, #15596). One ml of TRIzol solution was added to each tube containing the frozen tissue block and the tissue was homogenized by 10 passages through an 18 ½ G syringe needle. After centrifugation, the RNA was precipitated from the aqueous layer, washed and redissolved in RNase-free water. RNA concentration and the integrity of RNA were assessed by spectophotometry and gel electrophoresis. The RNA samples were stored at –80°C.

[84] Gene expression analyses were performed using the Affymetrix GeneChip System. The labeling of RNA samples, rat GeneChip (RG-U34A) hybridization and array scanning were carried out according to the Affymetrix GeneChip Expression Analysis Manual (r.4.0, 2000). Each animal's CA1 RNA was processed and run on a separate rat gene chip. Briefly, an average yield of 40 μg biotin-labeled cRNA target was obtained from 5 μg of total RNA from each CA1 sample, of which 20 ug cRNA was applied to one chip. The hybridization was run overnight in a rotating oven (Affymetrix) at 45^0C. The chips were then washed and stained on a fluidics station (Affymetrix) and scanned at a resolution of 3 μm in a confocal scanner (Agilent Affymetrix GeneArray Scanner).

[85] Each U34A rat chip (Affymetrix, Santa Clara, CA) contained 8,799 transcript probe sets (gene representations). Although the measured signal intensity for a transcript probe set (Methods) reflects mRNA content, it is referred to here as "gene expression". However, it is

well recognized that mRNA stability and other factors in addition to gene transcription can affect mRNA content.

[86] We used the microarray suite (MAS 4.0) software (Affymetrix) to calculate the overall noise of the image (the amount of variation around the mean intensity, Qraw) for each array. Overall noise was highly similar across arrays in all 3 age groups (Young: 21.81±1.55; Mid Aged: 21.25±2.24; Aged: 20.66±2.06, N.S.). "All probe set scaling" was used to set overall intensities of different arrays to an arbitrary target central intensity of 1500. Thus, the average intensity of each array was adjusted to the 1500 value using a scaling factor (SF). There was no significant difference in SF across ages (Young: 1.58±0.14; Mid Aged: 1.46±0.20; Aged: 1.63±0.16, N.S.).

[87] The algorithm used to determine Presence/Absence is listed in the Microarray Suite 4.0 Manual and is the basis upon which a particular transcript is determined to be reliably detectable by a given probe set. Average difference scores, the average of the difference in expression intensity (ADEI) of each probe pair within a probe set, formed the basis for determining expression (relative abundance) of transcripts, and throughout the text the term "expression level" refers to the ADEI score. When comparing across appropriately normalized arrays, the larger the ADEI score, the greater the relative expression for that particular message. However, ADEI scores are not comparable for relative expression levels among different messages on the same chip, as there are several other factors that can confound such an assessment (*e.g.*, p. 356, Affymetrix Microarray Suite 4.0 User's Guide).

[88] The Presence/Absence calls and Average Difference scores for all probe sets on all 29 arrays were then copied from the MAS pivot table to an Excel 9.0 (Microsoft, SR-1) workbook. From within Excel, the following data manipulations were performed.

[89] *Min-Max:* For the purposes of filtering (FIG. 2), each probe set was normalized according to the formula: $x' = \frac{x - \overline{X}\min}{X\max - X\min}$, where *x is ADEI score,* $\overline{X}_{min}$ is the mean for the age group with the lowest ADEI score, and $\overline{X}_{max}$ is the mean for the age group with the largest ADEI score. Thus, normalized mean values varied between 0 (lowest) and 1 (highest) for each probe set.

[90] *Standardization (Z-score)*: For the purpose of obtaining the mathematical means within functional categories and graphing, the data was normalized using the Z-score method:

$z = \frac{x - \overline{X}}{SD(x)}$, where $\overline{X}$ is the mean, and *SD(x)* is the standard deviation of ADEI across all age groups for an individual probe set.

[91] *Statistical Analysis.* All statistical tests were performed using a combination Excel (Microsoft, version 9, SR-1) and Sigma Stat (SPSS, version 2).

EXAMPLE 3

MULTI-STEP GENE IDENTIFICATION ALGORITHM

[92] The analytic algorithm of the invention, which addresses the bioinformatics issues noted above, comprise three main steps aimed first, at reducing the number of comparisons (to manage type I error), second, at reliably detecting modest aging differences with global statistical analyses (by ANOVA), and third, at identifying aging-related expression changes that were quantitatively correlated with cognitive function (by Pearson's test; Armitage P & Berry G *Statistical Methods in Medical Research, 2nd Edn.*, 200-205 (1987)) (*see*, FIG. 2).

[93] *Multiple Comparison Reduction Step.* The expected false positives in a series of multiple comparisons (false positive rate) are predicted to be a percentage of the total statistical comparisons to be made, as defined by the *p-value* (*i.e.*, tests at $p < 0.05$ will on average generate 5% false positives). Accordingly, the absolute numbers of expected false positives can be decreased simply by reducing the total transcript sets that are tested in a microarray analysis. This can be done by deleting all transcripts identified *a priori* as not likely to be relevant to the specific interests of the analysis.

[94] Using this step of the method, we reduced the total transcripts to be tested in three phases. In the first phase, we deleted quality control oligonucleotide sets ("control", $n = 60$) and all gene transcripts (probe sets) rated "absent" by our criteria. As used in this specification, the term "quality control oligonucleotides" are those oligonucleotides and polypeptides used to test for the appropriate behavior of the technological system, rather than to measure expression levels of biological interest.

[95] Of the original 8,799 sets, 4,118 gene transcript sets were removed at this stage, leaving 4,681 transcript sets that were called "present" for further consideration (FIG. 2, step 1a). In the second phase, we deleted all "present" transcript sets representing "expressed sequence tags" (ESTs), which have not yet been clearly linked to known genes (FIG. 2, step 1b). There were 1,213 such ESTs rated "present" that we filtered out in this phase, leaving

3,468 transcript sets for further consideration. The third reduction phase was based on our interest in persistent aging-dependent changes reflected in substantial differences between the youngest and oldest groups. We further decreased the total transcript sets to be tested by deleting sets in which the difference between the Young and the Aged group did not comprise at least 75% of the maximum normalized difference among groups (*i.e.*, in which age-related changes from the Young baseline values were maximal in the Mid-Aged group, but then reversed substantially (>25%) in the Aged group, possibly because of random, compensatory or developmental factors). There were 1,483 sets removed by this criterion, retaining 1,985 probe sets of the original 8,799 for formal statistical testing (FIG. 2; step 2). If the original 8,799 sets had been tested at the $p < .025$ alpha level, ~420 false positives would have been expected. However, by reducing the total number of sets to be tested for statistical significance (at $p < 0.025$), we reduced the absolute numbers of false positives expected from multiple tests, to ~50 (5% of 1985).

[96] *Group Statistical Testing Step (ANOVA).* In this second main step of the algorithm, each of the remaining1,985 transcript sets was tested by 1-way ANOVA for a significant effect of aging (at $p \leq 0.025$) across the 3 age groups (n = 9-10/group). Of the 1,985 tested sets, 233 were found to change significantly with aging (observed total positives). As noted, at $p < 0.025$, approximately 2.5% (~50) of the 1,985 tested should be significant by chance alone (expected false positives). In order to estimate the proportion of false discoveries anticipated among our 233 observed positives (*i.e.*, the fraction of observed positives expected to be false), we used the expected false positive value to calculate the false discovery rate (FDR) (Benjamini *et al.*, *Behav Brain Res 125*: 279-284 (2001)). For any multiple comparison, the false discovery rate provides an empirical estimate of the anticipated chance error rate among all positives detected. It is partly analogous to the *p value* of statistical tests, in that the false discovery rate yields the probability that any positive found at the alpha level used (in this case $p < 0.025$) is positive by chance alone.

[97] For the ANOVA-positive results, the FDR was 50/233 = 0.21, indicating that up to 21% of the observed positives might be positive by chance alone or, that any one positive had a 21% chance of being a false positive.

[98] In addition, we examined the FDR obtained using two other ANOVA *p-value* levels, $p < 0.01$ and $p < 0.001$. At the $p < .01$, ~ 20 genes should be found positive by chance alone among the 1,983 transcripts tested. A total of 145 total positives were observed, yielding an

FDR of 20/145 = 0.14. At $p < 0.001$ only 2 false positives are expected in 1,983 tests, and 70 total positives were found. This yields a FDR of 2/70 = .03. The latter, in particular, compares highly favorably with the 0.05 alpha level conventionally accepted for statistical significance in univariate analyses.

[99] However, as noted, additional confidence and validation is gained in microarray analyses when similar patterns of regulation are found among multiple functionally similar genes (Prolla *et al.*, *J Gerontol A Biol Sci Med Sci 56*: B327-330 (2001)). This is because such genes are not necessarily independent and their co-regulation can provide added cross-validation (*e.g.*, Mirnics *et al.*, *Neuron 28*: 53-67 (2000); Prolla *et al.*, *J Gerontol A Biol Sci Med Sci 56*: B327-330 (2001)). Consequently, in many cases, confidence advantages can be gained by relaxing *p-value* criteria in order to expand the numbers of genes included in functional categories. Mirnics K, *Nat Rev Neurosci 2:* 444-447 (2001). Further, relaxing stringency of the *p-value* reduces the likelihood of type II error (false negatives). Based on these rationales, we used the set of 233 genes obtained at the less stringent $p \leq 0.025$ alpha level (rather than the set of 70 at $p \leq 0.001$) for the next main step of our algorithm, the behavioral correlation analysis (FIG. 2, step 3a).

[100] *Cognitive Performance Correlation Step (Pearson's Test).* In this step we identify a specific subset of the 233 aging-significant (by ANOVA) genes that was also correlated with memory performance in both the OMT and SWM. We tested each of the 233 ANOVA-significant genes across animals for statistical correlation between that gene's expression value and behavioral scores (with Pearson's test).

[101] The expression of 161 of the 233 ANOVA-significant genes was correlated significantly with behavioral performance on memory-dependent tasks ($p \leq 0.025$; ACGs). Of these, 84 were significantly correlated with both OMT and SWM performance, 40 were significantly correlated with OMT, and 37 were significantly correlated with SMW (FIG. 2, step 3a). Of the 233 genes significant by ANOVA across age, 72 were significantly correlated with neither OMT nor SWM. Of these, 11 were significant by ANOVA across age at the more stringent p-value of $\leq .001$ (FIG. 2, step 3b) and were included for further analysis. Of the 161 ACGs, 64 exhibited decreased expression with aging and 97 exhibited increased expression with aging. Examples of the correlation patterns with behavior in the Aged group for the genes with the five highest correlations in each direction are shown in FIG. 3.

[102] Because of the voluminous literature involved, many relevant citations are not included here. In addition to the "dual function" status of some genes, the functions of many are not completely understood, and therefore, the categorization here, while generally consistent with published reports, is not definitive.

[103] ACGs that were downregulated with aging (TABLE 1A) appeared primarily to represent metabolic and neuronal functions. A substantial number of them fell into the category of oxidative metabolism (TABLE 1A). Many also fell into categories of synaptic/neuritic remodeling or other activity-dependent neuronal processes, *e.g.*, immediate early genes (IEGs) (TABLE 1A). Conversely, ACGs that were upregulated with aging fit primarily into categories that appeared to reflect activated inflammatory response (TABLE 1B).

[104] Additionally, among the main unexpected findings was a widespread upregulation in the expression of multiple genes encoding proteins for myelin synthesis (TABLE 1B) and lipid turnover (TABLE 1B). These various categories, overall, are consistent with a downward shift of oxidative metabolism in parallel with a major upregulation of lipid metabolism.

EXAMPLE 4

GENES IDENTIFIED BY THE METHOD OF THE INVENTION

[105] The following tables provide additional results from the tests performed above, and supplement the results presented in TABLES 1A and B.

TABLE 2

ESTs That Were Aging And Cognition Related or Showed Highly Significant Age-Dependent Changes in Expression Level

GenBank	Description	Young	Mid	Age	FC	ANOVA p
Decreased with Age						
	Correlated with both OMT and SWM					
AA963449	UI-R-E1-gj-e-08-0-UI.s1 cDNA	2499 ± 80	2122 ± 102	1874 ± 37	-1.33	0.0000
AA892532	EST196335 cDNA	4156 ± 85	4194 ± 80	3715 ± 100	-1.12	0.0010
AA859626	UI-R-E0-bs-h-02-0-UI.s1 cDNA	853 ± 22	705 ± 23	714 ± 35	-1.20	0.0013
AA893743	EST197546 cDNA	2292 ± 63	1985 ± 80	1846 ± 92	-1.24	0.0022
AI233365	EST230053 cDNA	8460 ± 232	7572 ± 289	7151 ± 226	-1.18	0.0042
H31665	EST105952 cDNA	1160 ± 56	1017 ± 34	942 ± 38	-1.23	0.0051
AA892353	ESTs, Moderately similar to JC5823 NADH dehydrogenase	890 ± 59	796 ± 66	602 ± 47	-1.48	0.0054
AI639247	mixed-tissue library cDNA clone rx03939 3	945 ± 36	814 ± 45	749 ± 36	-1.26	0.0063
AA858617	UI-R-E0-bq-b-06-0-UI.s1 cDNA	397 ± 17	294 ± 32	285 ± 22	-1.39	0.0072
AI639429	mixed-tissue library cDNA clone rx00973 3	341 ± 31	350 ± 22	252 ± 21	-1.35	0.0148
AA858620	UI-R-E0-bq-b-09-0-UI.s1 cDNA	153 ± 24	93 ± 10	86 ± 14	-1.78	0.0160
	Correlated with OMT					
AA866291	UI-R-A0-ac-e-12-0-UI.s3 cDNA	13818 ± 281	12477 ± 171	11987 ± 406	-1.15	0.0008
AA894104	EST197907 cDNA	5716 ± 164	5259 ± 156	4871 ± 179	-1.17	0.0060
AA799996	EST189493 cDNA	4881 ± 67	4812 ± 110	4407 ± 120	-1.11	0.0066
AA892805	EST196608 cDNA	6563 ± 147	6174 ± 247	5645 ± 212	-1.16	0.0176
AI639019	mixed-tissue library cDNA clone rx01107 3	353 ± 19	315 ± 24	265 ± 16	-1.33	0.0188
AA799538	EST189035 cDNA	1436 ± 156	1337 ± 76	963 ± 117	-1.49	0.0211

TABLE 2

ESTs That Were Aging And Cognition Related or Showed Highly Significant Age-Dependent Changes in Expression Level

GenBank	Description	Young	Mid	Age	FC	ANOVA p
Decreased with Age						
	Correlated with SWM					
AI070108	UI-R-Y0-lu-a-09-0-UI.s1 cDNA	1542 ± 36	1327 ± 39	1307 ± 58	-1.18	0.0022
AA866409	UI-R-E0-ch-a-03-0-UI.s1 cDNA	994 ± 38	814 ± 37	819 ± 35	-1.21	0.0026
AA859632	UI-R-E0-bs-h-08-0-UI.s1 cDNA	415 ± 53	352 ± 17	247 ± 18	-1.68	0.0040
AA891651	EST195454 cDNA	16635 ± 723	15405 ± 589	13530 ± 521	-1.23	0.0051
AA893032	ESTs, Moderately similar to CALX calnexin precursor	606 ± 26	491 ± 30	501 ± 17	-1.21	0.0060
AA891965	EST195768 cDNA	2353 ± 55	2260 ± 60	2088 ± 45	-1.13	0.0060
AA800708	ESTs, Weakly similar to S28312 hypothetical protein F02A9.4	1042 ± 38	945 ± 43	805 ± 58	-1.29	0.0065
AA964320	UI-R-C0-gu-e-09-0-UI.s1 cDNA	18110 ± 355	17683 ± 319	16605 ± 293	-1.09	0.0082
AA893173	EST196976 cDNA	9712 ± 294	8674 ± 503	8155 ± 222	-1.19	0.0196
H32977	EST108553 cDNA	3159 ± 74	2640 ± 85	2698 ± 66	-1.17	0.0001
AA874887	UI-R-E0-ci-g-10-0-UI.s1 cDNA	459 ± 43	284 ± 23	316 ± 11	-1.45	0.0004
AA850781	EST193549 cDNA	1886 ± 54	1570 ± 55	1602 ± 49	-1.18	0.0004
Increased with Age						
	Correlated with both OMT and SWM					
AI176456	ESTs, Weakly similar to endothelial actin-binding protein	8156 ± 447	9404 ± 462	12460 ± 511	1.53	0.0000
H31418	EST105434 cDNA	1176 ± 92	1530 ± 66	1904 ± 83	1.62	0.0000
AA858588	ESTs, Weakly similar to dihydrolipoamide acetyl transferase	2740 ± 80	2824 ± 86	3466 ± 198	1.26	0.0014
AA891785	EST195588 cDNA	1140 ± 122	1299 ± 82	1675 ± 89	1.47	0.0021
AA799803	ESTs, Weakly similar to K1CU cytoskeletal keratin (type 1)	149 ± 35	227 ± 28	297 ± 20	1.99	0.0035
AA799449	EST, Weakly similar to ubiquitin carboxyl-terminal hydrolase 4	-80 ± 7	-2 ± 26	17 ± 19	1.00	0.0044
	Correlated with OMT					
AA859777	UI-R-E0-bu-e-10-0-UI.s1 cDNA	1001 ± 43	1396 ± 76	1437 ± 87	1.44	0.0004
AI639532	mixed-tissue library cDNA clone rx01030 3	209 ± 16	282 ± 18	317 ± 22	1.52	0.0018
AA875059	UI-R-E0-cb-f-04-0-UI.s1 cDNA	233 ± 20	219 ± 12	297 ± 14	1.28	0.0023
AI012051	EST206502 cDNA	786 ± 68	987 ± 58	1200 ± 101	1.53	0.0042
AA800549	EST190046 cDNA	3647 ± 121	4078 ± 223	4573 ± 231	1.25	0.0132
	Correlated with SWM					
AA799854	EST189351 cDNA	211 ± 49	328 ± 46	487 ± 60	2.31	0.0037
AA892520	EST196323 cDNA	834 ± 38	826 ± 29	960 ± 36	1.15	0.0152
AA893607	EST197410 cDNA	-9 ± 19	69 ± 20	122 ± 22	1.99	0.0006
AI639381	mixed-tissue library cDNA clone rx01495 3	1531 ± 148	2417 ± 152	2353 ± 189	1.54	0.0013

TABLE 3

Genes and ESTs with Significant Age-Dependent Changes in Expression Level (ANOVA; $p \leq .05$) That Did Not Appear in TABLES 1 and 2

GenBank	Descriptions	Young	Mid	Age	FC	ANOVA p
Genes, Decreased						
	Correlate with both OMT and SWM					
M93273	somatostatin receptor subtype 2	1338 ± 142	1395 ± 105	1016 ± 30	-1.32	0.0252
AI175973	ESTs, Highly similar to NADH dehydrogenase	157 ± 18	136 ± 16	95 ± 14	-1.64	0.0314
AA799724	ESTs, Highly similar to DNA-directed RNA polymeraseI	2375 ± 47	2384 ± 79	2120 ± 91	-1.12	0.0321
X06769	FBJ v-fos oncogene homolog	1672 ± 156	1340 ± 154	1145 ± 79	-1.46	0.0329
X89696	TPCR06 protein	763 ± 50	625 ± 38	620 ± 35	-1.23	0.0361
D29766	v-crk-associated tyrosine kinase substrate	2478 ± 129	1929 ± 256	1568 ± 269	-1.58	0.0362
AI102839	cerebellar Ca-binding protein, spot 35 protein	2552 ± 110	2321 ± 131	2088 ± 110	-1.22	0.0364
M80550	adenylyl cyclase	6464 ± 207	6010 ± 212	5752 ± 133	-1.12	0.0403
U18771	Ras-related protein Rab-26	2631 ± 67	2373 ± 101	2350 ± 66	-1.12	0.0410
M36453	Inhibin, alpha	1438 ± 74	1350 ± 73	1178 ± 64	-1.22	0.0449
	Correlate with OMT					
AF055477	L-type voltage-dependent Ca^{2+} channel (α1D subunit)	2917 ± 144	2688 ± 119	2449 ± 74	-1.19	0.0275
AI013627	defender against cell death 1	10148 ± 175	9237 ± 310	9312 ± 219	-1.09	0.0289
AA891916	membrane interacting protein of RGS16	4586 ± 148	4330 ± 114	4117 ± 81	-1.11	0.0295
X67805	Synaptonemal complex protein 1	242 ± 22	189 ± 28	145 ± 23	-1.67	0.0319
D10874	lysosomal vacuolar proton pump (16 kDa)	23958 ± 745	21491 ± 849	21100 ± 812	-1.14	0.0436
D45247	proteasome subunit RCX	13926 ± 267	13333 ± 391	12526 ± 432	-1.11	0.0477
AF040954	putative protein phosphatase1 nuclear targeting subunit	1258 ± 27	1173 ± 35	1149 ± 28	-1.09	0.0515

TABLE 3

Genes and ESTs with Significant Age-Dependent Changes in Expression Level (ANOVA; $p \leq .05$) That Did Not Appear in TABLES 1 and 2

GenBank	Descriptions	Young	Mid	Age	FC	ANOVA p
Genes, Decreased						
	Correlate with SWM					
D10262	choline kinase	1248 ± 62	1092 ± 44	1079 ± 33	-1.16	0.0345
AI178921	Insulin degrading enzyme	174 ± 24	163 ± 9	111 ± 17	-1.56	0.0376
L29573	neurotransmitter transporter,noradrenalin	455 ± 47	342 ± 23	344 ± 31	-1.32	0.0475
	No significant behavioral correlations					
U75405	procollagen, type I, alpha 1	490 ± 18	378 ± 34	346 ± 22	-1.42	0.0017
L26292	Kruppel-like factor 4 (gut)	173 ± 21	100 ± 13	95 ± 10	-1.83	0.0018
AI169265	Atp6s1	18405 ± 380	16537 ± 447	16547 ± 318	-1.11	0.0027
L13202	RATHFH2 HNF-3/fork-head homolog-2 (HFH-2)	799 ± 63	557 ± 71	512 ± 19	-1.56	0.0027
AA799779	acyl-CoA:dihydroxyacetonephosphate acyltransferase	2742 ± 82	2363 ± 122	2181 ± 100	-1.26	0.0030
D89340	dipeptidylpeptidase III	2158 ± 76	1824 ± 68	1848 ± 64	-1.17	0.0038
AF019974	Chromogranin B, parathyroid secretory protein	10172 ± 290	8502 ± 400	8604 ± 334	-1.18	0.0038
U72620	Lot1	760 ± 52	620 ± 54	511 ± 35	-1.49	0.0042
U17254	immediate early gene transcription factor NGFI-B	3291 ± 202	2559 ± 115	2496 ± 180	-1.32	0.0045
M83745	Protein convertase subtilisin / kexin, type I	815 ± 43	630 ± 58	578 ± 39	-1.41	0.0048
AA893708	KIAA0560	2575 ± 62	2328 ± 84	2203 ± 74	-1.17	0.0061
H33725	associated molecule with the SH3 domain of STAM	1102 ± 26	970 ± 32	943 ± 41	-1.17	0.0064
AI230914	farnesyltransferase beta subunit	4044 ± 97	3465 ± 130	3498 ± 148	-1.16	0.0065
D37951	MIBP1 (c-myc intron binding protein 1)	6374 ± 194	5826 ± 173	5601 ± 100	-1.14	0.0067
AF076183	cytosolic sorting protein PACS-1a (PACS-1)	5098 ± 314	4039 ± 263	3774 ± 269	-1.35	0.0072
X82445	nuclear distribution gene C homolog (*Aspergillus*)	3311 ± 111	2910 ± 85	2901 ± 87	-1.14	0.0072
AA800948	Tuba4	8512 ± 215	7857 ± 402	6875 ± 342	-1.24	0.0076
D10699	ubiquitin carboxy-terminal hydrolase L1	19927 ± 1108	16996 ± 631	16532 ± 478	-1.21	0.0090
X57281	Glycine receptor alpha 2 subunit	199 ± 28	118 ± 19	111 ± 13	-1.79	0.0096
X76985	latexin	3937 ± 114	3187 ± 165	3332 ± 201	-1.18	0.0105
X84039	lumican	398 ± 30	283 ± 15	281 ± 36	-1.42	0.0109
U89905	alpha-methylacyl-CoA racemase	927 ± 39	793 ± 33	793 ± 27	-1.17	0.0110
M24852	Neuron specific protein PEP-19 (Purkinje cell protein 4)	6759 ± 349	5578 ± 280	5483 ± 310	-1.23	0.0146
U75917	clathrin-associated protein 17	6585 ± 232	5368 ± 330	5557 ± 291	-1.18	0.0158
X53427	glycogen synthase kinase 3 alpha (EC 2.7.1.37)	9799 ± 148	8843 ± 366	8572 ± 281	-1.14	0.0161
U28938	receptor-type protein tyrosine phosphatase D30	1564 ± 91	1354 ± 50	1286 ± 51	-1.22	0.0163
AA891880	Loc65042	2931 ± 59	2607 ± 85	2607 ± 98	-1.12	0.0171
AI232268	LDL receptor-related protein associated protein 1	1708 ± 68	1504 ± 59	1493 ± 36	-1.14	0.0186
AI045249	heat shock 70kD protein 8	537 ± 42	467 ± 46	366 ± 29	-1.47	0.0195
AF095927	protein phosphatase 2C	2968 ± 120	2516 ± 91	2549 ± 132	-1.16	0.0197
AA819708	Cox7a3	18590 ± 404	17401 ± 452	16742 ± 433	-1.11	0.0201
AA866257	ESTs	4750 ± 198	3994 ± 261	4021 ± 99	-1.18	0.0205
AA942685	cytosolic cysteine dioxygenase 1	9391 ± 397	8145 ± 443	7797 ± 325	-1.20	0.0221
D16478	mitochondrial long-chain enoyl-CoA hydratase	3913 ± 78	3615 ± 95	3499 ± 118	-1.12	0.0222
D88586	eosinophil cationic protein	2522 ± 108	2236 ± 206	1853 ± 138	-1.36	0.0226

TABLE 3

Genes and ESTs with Significant Age-Dependent Changes in Expression Level (ANOVA; $p \leq .05$) That Did Not Appear in TABLES 1 and 2

GenBank	Descriptions	Young	Mid	Age	FC	ANOVA p
Genes, Decreased						
	No significant behavioral correlations					
E03229	cytosolic cysteine dioxygenase 1	5634 ± 433	4518 ± 512	3918 ± 238	-1.44	0.0227
AB006451	Tim23	5968 ± 155	5562 ± 198	5315 ± 100	-1.12	0.0241
M10068	NADPH-cytochrome P-450 oxidoreductase	5771 ± 205	4998 ± 190	5139 ± 191	-1.12	0.0242
Z48225	protein synthesis initiation factor eIF-2B delta subunit	2710 ± 114	2415 ± 96	2327 ± 78	-1.16	0.0260
M93669	Secretogranin II	4917 ± 225	4395 ± 136	4309 ± 105	-1.14	0.0266
U17254	immediate early gene transcription factor NGFI-B	6004 ± 635	4395 ± 228	4694 ± 316	-1.28	0.0269
U38801	DNA polymerase beta	1173 ± 61	1001 ± 45	997 ± 39	-1.18	0.0270
AA874874	ESTs, Highly similar to alcohol dehydrogenase class III	3683 ± 64	3429 ± 83	3436 ± 60	-1.07	0.0278
AB016532	period homolog 2 (Drosophila)	1440 ± 117	1116 ± 84	1135 ± 62	-1.27	0.0290
AF007758	synuclein, alpha	17737 ± 473	15958 ± 751	15463 ± 459	-1.15	0.0295
U04738	Somatostatin receptor subtype 4	2066 ± 109	1680 ± 70	1733 ± 122	-1.19	0.0300
AF007890	resection-induced TPI (rs11)	513 ± 48	388 ± 43	326 ± 50	-1.58	0.0307
AA874969	ESTs, Highly similar to c-Jun leucine zipper interactive	8555 ± 211	7333 ± 326	7531 ± 387	-1.14	0.0310
M31174	thyroid hormone receptor alpha	16273 ± 775	14217 ± 473	14395 ± 419	-1.13	0.0312
AA801286	Inositol (myo)-1(or 4)-monophosphatase 1	4767 ± 151	4270 ± 199	4155 ± 118	-1.15	0.0312
AF007554	Mucin1	385 ± 29	276 ± 35	282 ± 26	-1.37	0.0316
X98399	solute carrier family 14, member 1	2002 ± 105	1555 ± 95	1615 ± 151	-1.24	0.0329
AI168942	branched chain keto acid dehydrogenase E1	1580 ± 73	1367 ± 58	1418 ± 30	-1.11	0.0334
AF023087	Early growth response 1	20068 ± 1720	16426 ± 661	16294 ± 622	-1.23	0.0339
K02248	Somatostatin	4314 ± 165	3565 ± 189	3651 ± 245	-1.18	0.0341
AA859954	Vacuole Membrane Protein 1	4197 ± 122	3755 ± 119	3789 ± 128	-1.11	0.0346
AI176621	iron-responsive element-binding protein	1505 ± 66	1334 ± 63	1287 ± 42	-1.17	0.0348
AI010110	SH3-domain GRB2-like 1	1981 ± 67	1596 ± 113	1669 ± 117	-1.19	0.0363
L42855	transcription elongation factor B (SIII) polypeptide 2	10836 ± 201	9654 ± 417	9859 ± 283	-1.10	0.0368
AI136891	zinc finger protein 36, C3H type-like 1	3892 ± 153	3427 ± 188	3247 ± 160	-1.20	0.0369
S77492	Bone morphogenetic protein 3	123 ± 15	103 ± 17	65 ± 14	-1.89	0.0374
AI230778	ESTs, Highly similar to protein-tyrosine sulfotrans. 2	2049 ± 41	2019 ± 120	1714 ± 101	-1.20	0.0380
AA859980	T-complex 1	1710 ± 77	1411 ± 71	1478 ± 90	-1.16	0.0383
U27518	UDP-glucuronosyltransferase	316 ± 22	266 ± 26	223 ± 24	-1.42	0.0394
AF030088	RuvB-like protein 1	497 ± 151	252 ± 39	181 ± 21	-2.74	0.0398
AF013144	MAP-kinase phosphatase (cpg21)	1551 ± 185	1100 ± 98	1149 ± 92	-1.35	0.0408
M58404	thymosin, beta 10	20359 ± 853	18136 ± 773	17948 ± 400	-1.13	0.0413
AA819500	ESTs, Highly similar to AC12_HUMAN 37 kD subunit	532 ± 44	434 ± 30	411 ± 26	-1.29	0.0417
AF020046	integrin alpha E1, epithelial-associated	113 ± 17	109 ± 12	70 ± 10	-1.62	0.0419
D10854	aldehyde reductase	18091 ± 526	16744 ± 433	16538 ± 354	-1.09	0.0422
AF000899	p58/p45, nucleolin	1666 ± 114	1381 ± 81	1359 ± 73	-1.23	0.0430
S77858	non-muscle myosin alkali light chain	10848 ± 292	9865 ± 409	9642 ± 278	-1.12	0.0435
J05031	Isovaleryl Coenzyme A dehydrogenase	1996 ± 57	1799 ± 75	1792 ± 45	-1.11	0.0451

TABLE 3
Genes and ESTs with Significant Age-Dependent Changes in Expression Level
(ANOVA; $p \leq .05$) That Did Not Appear in TABLES 1 and 2

GenBank	Descriptions	Young	Mid	Age	FC	ANOVA p
Genes, Decreased						
	No significant behavioral correlations					
J02773	heart fatty acid binding protein	2242 ± 88	1918 ± 118	1885 ± 99	-1.19	0.0453
AA891041	jun B proto-oncogene	1125 ± 128	788 ± 79	871 ± 68	-1.29	0.0453
AA817887	profilin	12549 ± 398	10859 ± 592	10886 ± 498	-1.15	0.0460
U38379	Gamma-glutamyl hydrolase	2340 ± 215	2136 ± 177	1693 ± 141	-1.38	0.0467
D78308	calreticulin	8256 ± 349	7233 ± 343	7446 ± 126	-1.11	0.0486
AA818487	cyclophilin B	8861 ± 410	7912 ± 293	7779 ± 236	-1.14	0.0488
AA799479	ESTs, Highly similar to NADH-ubiquinone oxidoreduct.	4937 ± 203	4124 ± 291	4075 ± 263	-1.21	0.0496
AI104388	heat shock 27kD protein 1	2102 ± 72	2072 ± 81	1839 ± 82	-1.14	0.0511
X59737	ubiquitous mitochondrial creatine kinase	11016 ± 315	9658 ± 360	9950 ± 451	-1.11	0.0512
D83948	adult liver S1-1 protein	1411 ± 45	1249 ± 78	1221 ± 30	-1.16	0.0522
AA893788	ESTs, Highly similar to chromobox protein homolog 5	658 ± 33	562 ± 23	568 ± 31	-1.16	0.0541
Genes, Increased						
	Correlate with both OMT and SWM					
AI230247	selenoprotein P, plasma, 1	7467 ± 279	8179 ± 312	8700 ± 319	1.17	0.0304
AF016269	kallikrein 6 (neurosin, zyme)	1141 ± 75	1166 ± 51	1375 ± 72	1.21	0.0353
AF021935	Ser-Thr protein kinase	2 ± 111	453 ± 193	649 ± 184	10.63	0.0395
M24104	synaptobrevin 2	1145 ± 55	1783 ± 260	1794 ± 210	1.57	0.0544
	Correlate with OMT					
AI235344	geranylgeranyltransferase type I (GGTase-I)	336 ± 21	362 ± 16	413 ± 21	1.23	0.0310
X60212	ASI homolog of bacterial ribosomal subunit protein L22	17230 ± 994	18514 ± 1115	21606 ± 1305	1.25	0.0365
U14950	tumor suppressor homolog (synapse associ. protein)	315 ± 29	507 ± 61	498 ± 64	1.58	0.0379
X53504	ribosomal protein L12	9290 ± 179	9922 ± 247	10210 ± 290	1.10	0.0448
AA955388	Na^+K^+ transporting ATPase 2, beta polypeptide 2	2361 ± 155	2863 ± 320	3237 ± 170	1.37	0.0451
X76489	CD9 cell surface glycoprotein	2485 ± 199	2713 ± 135	3106 ± 170	1.25	0.0467
D28110	myelin-associated oligodendrocytic basic protein	5947 ± 490	7855 ± 539	8814 ± 1109	1.48	0.0499
	Correlate with SWM					
U10357	pyruvate dehydrogenase kinase 2 subunit p45 (PDK2)	3565 ± 133	3921 ± 274	4485 ± 240	1.26	0.0292
D00569	2,4-dienoyl CoA reductase 1, mitochondrial	200 ± 22	241 ± 32	307 ± 24	1.54	0.0293
AA818240	Nuclear pore complex protein	308 ± 35	440 ± 42	424 ± 28	1.38	0.0329
M24104	synaptobrevin 2	685 ± 193	1379 ± 247	1581 ± 250	2.31	0.0332
D28557	cold shock domain protein A	1383 ± 89	1491 ± 129	1803 ± 106	1.30	0.0337
X54467	cathepsin D	3715 ± 294	4091 ± 388	5138 ± 431	1.38	0.0373
X13905	ras-related rab1B protein	201 ± 111	803 ± 179	689 ± 181	3.43	0.0388
AI228548	ESTs, Highly similar to DKFZp586G0322.1	1909 ± 140	2053 ± 75	2321 ± 110	1.22	0.0412
V01244	Prolactin	75 ± 37	70 ± 37	354 ± 140	4.75	0.0476
L24896	glutathione peroxidase 4	12303 ± 650	12725 ± 456	14045 ± 358	1.14	0.0479

TABLE 3

Genes and ESTs with Significant Age-Dependent Changes in Expression Level (ANOVA; $p \leq .05$) That Did Not Appear in TABLES 1 and 2

GenBank	Descriptions	Young	Mid	Age	FC	ANOVA p
Genes, Increased						
	No significant behavioral correlations					
U77777	interleukin 18	252 ± 15	290 ± 12	371 ± 32	1.47	0.0025
AI102299	Bid3	267 ± 98	527 ± 59	603 ± 21	2.26	0.0032
L19998	Phenol-preferring sulfotransferase 1A	373 ± 36	507 ± 27	616 ± 69	1.65	0.0065
AF051561	solute carrier family 12, member 2	2749 ± 82	3228 ± 83	3281 ± 163	1.19	0.0074
U08259	Glutamate receptor, N-methyl D-aspartate 2C	919 ± 34	989 ± 49	1118 ± 38	1.22	0.0074
AB008538	HB2	3733 ± 133	4436 ± 189	4264 ± 117	1.14	0.0087
AF016296	neuropilin	1838 ± 121	2279 ± 85	2259 ± 110	1.23	0.0111
X62950	pBUS30 with repetitive elements	360 ± 25	577 ± 67	548 ± 47	1.52	0.0124
AF030050	replication factor C	857 ± 62	1154 ± 73	1148 ± 81	1.34	0.0127
AA848831	lysophosphatidic acid G-protein-coupled receptor, 2	1854 ± 170	2729 ± 225	2784 ± 261	1.50	0.0129
M91234	VL30 element	2573 ± 152	3409 ± 221	3467 ± 254	1.35	0.0134
J05132	UDP-glucuronosyltransferase	968 ± 76	1283 ± 68	1212 ± 74	1.25	0.0148
AF008554	implantation-associated protein (IAG2)	362 ± 46	528 ± 33	500 ± 40	1.38	0.0162
AI231807	ferritin light chain 1	5496 ± 174	5863 ± 273	6469 ± 197	1.18	0.0163
S72594	tissue inhibitor of metalloproteinase 2	3615 ± 205	4386 ± 216	4227 ± 114	1.17	0.0170
S61868	Ryudocan/syndecan 4	6117 ± 292	6315 ± 211	7348 ± 385	1.20	0.0182
X06916	S100 calcium-binding protein A4	572 ± 40	630 ± 60	868 ± 99	1.52	0.0184
U67136	A kinase (PRKA) anchor protein 5	306 ± 59	531 ± 66	551 ± 61	1.80	0.0191
Y17295	thiol-specific antioxidant protein (1-Cys peroxiredoxin)	2414 ± 154	3037 ± 133	2998 ± 193	1.24	0.0221
D45249	protease (prosome, macropain) 28 subunit, alpha	4169 ± 119	4657 ± 205	4808 ± 121	1.15	0.0223
U67137	guanylate kinase associated protein	3198 ± 366	4262 ± 333	4338 ± 177	1.36	0.0229
AF074608	MHC class I antigen (RT1.EC2) gene	782 ± 129	940 ± 110	1213 ± 69	1.55	0.0231
U67080	r-MyT13	-29 ± 17	74 ± 38	92 ± 32	1.50	0.0250
AI013861	3-hydroxyisobutyrate dehydrogenase	3347 ± 136	3759 ± 101	3678 ± 73	1.10	0.0255
S53527	S100 calcium-binding protein, beta (neural)	25683 ± 925	25830 ± 765	29195 ± 1184	1.14	0.0266
D89730	Fibulin 3, fibulin-like extracellular matrix protein 1	239 ± 23	351 ± 52	424 ± 50	1.78	0.0271
D90211	Lysosomal-associated membrane protein 2	3095 ± 142	3577 ± 157	3715 ± 168	1.20	0.0276
AA859645	attractin	2647 ± 81	2871 ± 82	2942 ± 60	1.11	0.0278
X55153	ribosomal protein P2	18829 ± 779	19676 ± 485	21368 ± 641	1.13	0.0284
M55015	nucleolin	6685 ± 139	6738 ± 263	7385 ± 147	1.10	0.0297
L25605	Dynamin 2	759 ± 84	780 ± 71	1109 ± 129	1.46	0.0303
AI231807	ferritin light chain 1	9399 ± 508	10459 ± 538	11268 ± 329	1.20	0.0312
L00191	Fibronectin 1	395 ± 23	530 ± 44	557 ± 53	1.41	0.0316
D28110	myelin-associated oligodendrocytic basic protein	837 ± 127	1177 ± 106	1331 ± 141	1.59	0.0320
AI176595	Cathepsin L	2414 ± 73	2639 ± 57	2678 ± 80	1.11	0.0324
X14323	Fc receptor, IgG, alpha chain transporter	431 ± 38	510 ± 71	640 ± 42	1.49	0.0328
X74226	LL5 protein	2042 ± 69	2000 ± 66	2279 ± 92	1.12	0.0330
AA892775	Lysozyme	1760 ± 88	1781 ± 65	2438 ± 314	1.39	0.0337
X02904	glutathione S-transferase P subunit	2861 ± 124	3514 ± 276	3570 ± 141	1.25	0.0339
AI012589	glutathione S-transferase, pi 2	6325 ± 340	7706 ± 465	7807 ± 418	1.23	0.0353
AB000778	Phoshpolipase D gene 1	194 ± 24	270 ± 18	287 ± 31	1.48	0.0374

TABLE 3

Genes and ESTs with Significant Age-Dependent Changes in Expression Level (ANOVA; $p \leq .05$) That Did Not Appear in TABLES 1 and 2

GenBank	Descriptions	Young	Mid	Age	FC	ANOVA p
Genes, Increased						
	No significant behavioral correlations					
X97443	integral membrane protein Tmp21-I (p23)	862 ± 64	1194 ± 131	1211 ± 90	1.40	0.0396
X58294	carbonic anhydrase 2	5372 ± 252	6554 ± 399	6347 ± 290	1.18	0.0398
M99485	Myelin oligodendrocyte glycoprotein	2546 ± 107	2645 ± 113	3176 ± 259	1.25	0.0405
M23601	Monoamine oxidase B	4962 ± 268	5244 ± 152	5763 ± 212	1.16	0.0406
J05022	peptidylarginine deiminase	3834 ± 133	4231 ± 137	4503 ± 231	1.17	0.0425
Z49858	plasmolipin	2111 ± 146	2437 ± 69	2624 ± 172	1.24	0.0429
D17309	delta 4-3-ketosteroid-5-beta-reductase	568 ± 66	930 ± 96	951 ± 150	1.67	0.0432
AA955306	ras-related protein rab10	3912 ± 289	4796 ± 339	4975 ± 257	1.27	0.0444
M19936	Prosaposin- sphingolipid hydrolase activator	12981 ± 997	14182 ± 780	16095 ± 751	1.24	0.0463
M57276	Leukocyte antigen (Ox-44)	879 ± 79	1071 ± 65	1117 ± 57	1.27	0.0469
J02752	acyl-coA oxidase	1853 ± 119	2187 ± 155	2344 ± 118	1.26	0.0470
U78517	cAMP-regulated guanine nucleotide exchange factor II	3400 ± 134	3956 ± 216	3903 ± 113	1.15	0.0477
AI102031	myc box dependent interacting protein 1	6381 ± 242	6919 ± 237	7265 ± 236	1.14	0.0486
M89646	ribosomal protein S24	14041 ± 448	15044 ± 319	15482 ± 416	1.10	0.0491
AA924925	ER transmembrane protein Dri 42	435 ± 209	799 ± 143	1067 ± 160	2.45	0.0493
X16933	RNA binding protein p45AUF1	1516 ± 166	2186 ± 203	2139 ± 221	1.41	0.0499
X72757	cox VIa gene (liver)	666 ± 73	855 ± 39	829 ± 51	1.24	0.0502
AA957132	N-acetylglucosaminyltransferase I	242 ± 26	401 ± 56	398 ± 54	1.64	0.0508
AA818025	CD59 antigen	5668 ± 298	6175 ± 280	6909 ± 414	1.22	0.0509
AI237007	ESTs, Highly similar to flavoprot.-ubiquin. Oxidoreduct.	48 ± 37	117 ± 50	195 ± 29	3.19	0.0519
U07619	Coagulation factor III (thromboplastin, tissue factor)	701 ± 37	792 ± 37	847 ± 46	1.21	0.0544
ESTs, Decreased						
	Correlate with both OMT and SWM					
AA874830	UI-R-E0-cg-f-04-0-UI.s1 cDNA	1584 ± 87	1406 ± 65	1323 ± 33	-1.20	0.0268
AA875032	UI-R-E0-cb-h-09-0-UI.s1 cDNA	1770 ± 40	1536 ± 91	1490 ± 72	-1.19	0.0288
AA799599	EST189096 cDNA	6628 ± 210	6184 ± 281	5618 ± 257	-1.18	0.0328
AA892813	EST196616 cDNA	218 ± 41	241 ± 54	92 ± 25	-2.37	0.0363
AA799529	EST189026 cDNA	1590 ± 61	1529 ± 51	1388 ± 55	-1.15	0.0466
AA893584	EST197387 cDNA	4021 ± 120	3570 ± 206	3416 ± 167	-1.18	0.0548
	Correlate with OMT					
AA894305	EST198108 cDNA	4779 ± 107	4393 ± 138	4261 ± 151	-1.12	0.0349
AA800622	EST190119 cDNA	2372 ± 76	2325 ± 102	2056 ± 83	-1.15	0.0370
AA893690	EST197493 cDNA	5102 ± 229	4813 ± 146	4334 ± 220	-1.18	0.0378
AA891221	EST195024 cDNA	4562 ± 179	4159 ± 173	3956 ± 128	-1.15	0.0423
AA893320	EST197123 cDNA	1110 ± 35	1071 ± 69	911 ± 57	-1.22	0.0455
AA891537	EST195340 cDNA	2420 ± 94	2098 ± 85	2145 ± 96	-1.13	0.0468
AA799680	EST189177 cDNA	560 ± 45	544 ± 33	431 ± 39	-1.30	0.0504

TABLE 3

Genes and ESTs with Significant Age-Dependent Changes in Expression Level (ANOVA; $p \leq .05$) That Did Not Appear in TABLES 1 and 2

GenBank	Descriptions	Young	Mid	Age	FC	ANOVA p
ESTs, Decreased						
	Correlate with SWM					
AA893199	EST197002 cDNA	2422 ± 100	2482 ± 67	2129 ± 112	-1.14	0.0287
AA799636	EST189133 cDNA	3279 ± 92	2986 ± 125	2826 ± 124	-1.16	0.0358
AA874995	UI-R-E0-cf-d-08-0-UI.s1 cDNA	1202 ± 44	1123 ± 37	1068 ± 19	-1.13	0.0360
AA892298	EST196101 cDNA	302 ± 26	243 ± 13	229 ± 22	-1.32	0.0456
AA892538	EST196341 cDNA	1033 ± 64	902 ± 41	868 ± 36	-1.19	0.0547
	No significant behavioral correlations					
AA859690	UI-R-E0-bx-e-11-0-UI.s1 cDNA	297 ± 29	173 ± 40	137 ± 10	-2.17	0.0017
AA875004	UI-R-E0-cb-b-07-0-UI.s1 cDNA	965 ± 40	774 ± 44	776 ± 30	-1.24	0.0022
AA891037	EST194840 cDNA	2174 ± 98	1781 ± 83	1774 ± 68	-1.23	0.0031
AA893185	EST196988 cDNA	7616 ± 301	6680 ± 137	6666 ± 166	-1.14	0.0045
AA892511	EST196314 cDNA	4716 ± 113	4061 ± 150	4216 ± 139	-1.12	0.0068
AA875129	UI-R-E0-bu-e-01-0-UI.s2 cDNA	1214 ± 28	1093 ± 33	1062 ± 34	-1.14	0.0071
AA800693	EST190190 cDNA	3177 ± 84	2844 ± 82	2830 ± 71	-1.12	0.0072
AA859562	UI-R-E0-bv-b-03-0-UI.s1 cDNA	933 ± 91	682 ± 57	606 ± 58	-1.54	0.0078
AA860030	UI-R-E0-bz-e-07-0-UI.s2 cDNA	20727 ± 774	17601 ± 811	17941 ± 508	-1.16	0.0090
AA891727	EST195530 cDNA	5801 ± 266	4821 ± 204	5038 ± 189	-1.15	0.0114
AA892796	EST196599 cDNA	6952 ± 143	6326 ± 167	6441 ± 110	-1.08	0.0117
AI639477	mixed-tissue library cDNA clone rx02351 3	264 ± 26	193 ± 53	78 ± 40	-3.39	0.0154
AA893717	EST197520 cDNA	515 ± 24	442 ± 35	386 ± 27	-1.33	0.0179
AA892414	EST196217 cDNA	2935 ± 143	2507 ± 111	2511 ± 79	-1.17	0.0185
AA893743	EST197546 cDNA	2730 ± 120	2282 ± 121	2181 ± 154	-1.25	0.0193
AI176491	EST220076 cDNA	5180 ± 182	4665 ± 213	4450 ± 108	-1.16	0.0199
AA799481	EST188978 cDNA	1036 ± 33	889 ± 31	916 ± 44	-1.13	0.0240
AA859643	UI-R-E0-bs-a-08-0-UI.s1 cDNA	4772 ± 162	3978 ± 177	4165 ± 238	-1.15	0.0252
AA875257	UI-R-E0-cq-d-12-0-UI.s1 cDNA	1715 ± 133	1369 ± 92	1342 ± 71	-1.28	0.0255
AA685974	EST108806 cDNA	5543 ± 142	4855 ± 194	4974 ± 184	-1.11	0.0275
AA891476	EST195279 cDNA	7512 ± 289	7075 ± 235	6520 ± 208	-1.15	0.0279
AA891950	EST195753 cDNA	865 ± 18	818 ± 45	725 ± 33	-1.19	0.0284
AA875019	UI-R-E0-cb-f-08-0-UI.s1 cDNA	1007 ± 32	908 ± 29	901 ± 29	-1.12	0.0357
AA866477	UI-R-E0-br-h-03-0-UI.s1 cDNA	11037 ± 230	9932 ± 341	10208 ± 283	-1.08	0.0376
AI639209	mixed-tissue library cDNA clone rx00680 3	763 ± 57	820 ± 98	562 ± 44	-1.36	0.0385
AI102868	EST212157 cDNA	11364 ± 316	9876 ± 516	9787 ± 490	-1.16	0.0418
AI178204	EST221869 cDNA	2465 ± 180	2162 ± 137	1905 ± 122	-1.29	0.0419
AA799858	EST189355 cDNA	1068 ± 76	925 ± 58	827 ± 58	-1.29	0.0427
AA800026	EST189523 cDNA	249 ± 29	155 ± 26	144 ± 35	-1.73	0.0429
AA892637	EST196440 cDNA	809 ± 16	757 ± 24	739 ± 16	-1.10	0.0430
AA859545	ESTs, Weakly similar to hypothetical protein C09H6.3	3289 ± 167	2762 ± 137	2876 ± 134	-1.14	0.0442
AA859848	UI-R-E0-cc-h-10-0-UI.s1 cDNA	3396 ± 315	3150 ± 165	2626 ± 129	-1.29	0.0456
H33086	EST108750 cDNA	21205 ± 763	18706 ± 530	19138 ± 810	-1.11	0.0477
AA893224	EST197027 cDNA	2325 ± 67	2150 ± 75	2076 ± 64	-1.12	0.0502

TABLE 3
Genes and ESTs with Significant Age-Dependent Changes in Expression Level (ANOVA; $p \leq .05$) That Did Not Appear in TABLES 1 and 2

GenBank	Descriptions	Young	Mid	Age	FC	ANOVA p
ESTs, Increased						
	Correlate with both OMT and SWM					
AA893946	EST197749 cDNA	371 ± 45	565 ± 43	544 ± 72	1.47	0.0440
	Correlate with OMT					
AI638997	mixed-tissue library cDNA clone rx05048 3	402 ± 23	450 ± 26	483 ± 11	1.20	0.0381
AI177404	EST221024 cDNA	1012 ± 46	1193 ± 73	1245 ± 65	1.23	0.0429
	Correlate with SWM					
AA800318	EST189815 cDNA	315 ± 46	376 ± 40	474 ± 41	1.51	0.0421
	No significant behavioral correlations					
AA893082	EST196885 cDNA	1454 ± 95	1902 ± 43	1865 ± 110	1.28	0.0021
AA892986	EST196789 cDNA	586 ± 19	627 ± 33	756 ± 39	1.29	0.0025
M13100	long interspersed repetitive DNA sequence LINE3	4328 ± 230	5963 ± 252	5947 ± 457	1.37	0.0026
AA891734	EST195537 cDNA	1648 ± 86	1778 ± 82	2045 ± 60	1.24	0.0037
AI171966	ESTs, Highly similar to selenide, water dikinase 2	880 ± 42	934 ± 30	1181 ± 93	1.34	0.0049
AI639151	mixed-tissue library cDNA clone rx02802 3	939 ± 49	1192 ± 80	1223 ± 54	1.30	0.0083
AA875037	UI-R-E0-cb-a-03-0-UI.s1 cDNA	11 ± 71	268 ± 70	357 ± 78	5.84	0.0084
AA891690	ESTs, Weakly similar to p-serine aminotransferase	1858 ± 76	1955 ± 65	2296 ± 131	1.24	0.0088
AA891810	EST195613 cDNA	1504 ± 140	2028 ± 155	2274 ± 202	1.51	0.0125
AA866432	UI-R-E0-ch-e-06-0-UI.s1 cDNA	2777 ± 150	3380 ± 102	3493 ± 226	1.26	0.0143
X05472	2.4 kb repeat DNA right terminal region	4188 ± 565	5325 ± 564	7241 ± 899	1.73	0.0173
AA892146	EST195949 cDNA	5386 ± 450	7073 ± 436	7004 ± 418	1.30	0.0187
AA852046	EST194815 cDNA	1697 ± 140	2163 ± 92	2051 ± 112	1.21	0.0234
AA799396	EST188893 cDNA	163 ± 26	264 ± 35	269 ± 24	1.65	0.0275
AI638971	mixed-tissue library cDNA clone rx04989 3	128 ± 26	188 ± 13	213 ± 24	1.67	0.0285
AA892520	EST196323 cDNA	479 ± 31	526 ± 28	601 ± 33	1.25	0.0305
AA891774	EST195577 cDNA	-518 ± 92	-115 ± 126	-147 ± 108	1.00	0.0322
M13100	long interspersed repetitive DNA sequence LINE3	8845 ± 982	12115 ± 1117	12282 ± 814	1.39	0.0366
AI639257	mixed-tissue library cDNA clone rx01119 3	172 ± 23	306 ± 41	286 ± 41	1.66	0.0386
AA866299	UI-R-A0-ac-f-12-0-UI.s3 cDNA	684 ± 45	810 ± 24	885 ± 73	1.29	0.0390
AA799773	EST189270 cDNA	299 ± 30	408 ± 24	433 ± 50	1.45	0.0407
AA866299	UI-R-A0-ac-f-12-0-UI.s3 cDNA	522 ± 32	623 ± 28	626 ± 31	1.20	0.0415
AA891944	EST195747 cDNA	193 ± 15	198 ± 13	247 ± 20	1.28	0.0488

[106] Using the method of the invention, we have identified a set of genes and ESTs that changed with age by ANOVA ($p \leq .05$), but which are not ACGs. These include AA685974 (EST108806 cDNA); AA799396 (EST188893 cDNA); AA799479 (ESTs, Highly similar to NADH-ubiquinone oxidoreduct.); AA799481 (EST188978 cDNA); AA799529 (EST189026 cDNA); AA799599 (EST189096 cDNA); AA799636 (EST189133 cDNA); AA799680 (EST189177 cDNA); AA799724 (ESTs, Highly similar to DNA-directed RNA polymeraseI); AA799773 (EST189270 cDNA); AA799779 (acyl-CoA:dihydroxyacetonephosphate acyltransferase); AA799858 (EST189355 cDNA); AA800026 (EST189523 cDNA); AA800318 (EST189815 cDNA); AA800622 (EST190119 cDNA); AA800693 (EST190190 cDNA); AA800948 (Tuba4); AA801286 (Inositol (myo)-1(or 4)-monophosphatase 1); AA817887 (profilin); AA818025 (CD59 antigen); AA818240 (Nuclear pore complex

protein); AA818487 (cyclophilin B); AA819500 (ESTs, Highly similar to AC12_HUMAN 37 kD subunit); AA819708 (Cox7a3); AA848831 (lysophosphatidic acid G-protein-coupled receptor, 2); AA852046 (EST194815 cDNA); AA859545 (ESTs, Weakly similar to hypothetical protein C09H6.3); AA859562 (UI-R-E0-bv-b-03-0-UI.s1 cDNA); AA859643 (UI-R-E0-bs-a-08-0-UI.s1 cDNA); AA859645 (attractin); AA859690 (UI-R-E0-bx-e-11-0-UI.s1 cDNA); AA859848 (UI-R-E0-cc-h-10-0-UI.s1 cDNA); AA859954 (Vacuole Membrane Protein 1); AA859980 (T-complex 1); AA860030 (UI-R-E0-bz-e-07-0-UI.s2 cDNA); AA866257 (ESTs); AA866299 (UI-R-A0-ac-f-12-0-UI.s3 cDNA); AA866299 (UI-R-A0-ac-f-12-0-UI.s3 cDNA); AA866432 (UI-R-E0-ch-e-06-0-UI.s1 cDNA); AA866477 (UI-R-E0-br-h-03-0-UI.s1 cDNA); AA874830 (UI-R-E0-cg-f-04-0-UI.s1 cDNA); AA874874 (ESTs, Highly similar to alcohol dehydrogenase class III); AA874969 (ESTs, Highly similar to c-Jun leucine zipper interactive); AA874995 (UI-R-E0-cf-d-08-0-UI.s1 cDNA); AA875004 (UI-R-E0-cb-b-07-0-UI.s1 cDNA); AA875019 (UI-R-E0-cb-f-08-0-UI.s1 cDNA); AA875032 (UI-R-E0-cb-h-09-0-UI.s1 cDNA); AA875037 (UI-R-E0-cb-a-03-0-UI.s1 cDNA); AA875129 (UI-R-E0-bu-e-01-0-UI.s2 cDNA); AA875257 (UI-R-E0-cq-d-12-0-UI.s1 cDNA); AA891037 (EST194840 cDNA); AA891041 (jun B proto-oncogene); AA891221 (EST195024 cDNA); AA891476 (EST195279 cDNA); AA891537 (EST195340 cDNA); AA891690 (ESTs, Weakly similar to p-serine aminotransferase); AA891727 (EST195530 cDNA); AA891734 (EST195537 cDNA); AA891774 (EST195577 cDNA); AA891810 (EST195613 cDNA); AA891880 (Loc65042); AA891916 (membrane interacting protein of RGS16); AA891944 (EST195747 cDNA); AA891950 (EST195753 cDNA); AA892146 (EST195949 cDNA); AA892298 (EST196101 cDNA); AA892414 (EST196217 cDNA); AA892511 (EST196314 cDNA); AA892520 (EST196323 cDNA); AA892538 (EST196341 cDNA); AA892637 (EST196440 cDNA); AA892775 (Lysozyme); AA892796 (EST196599 cDNA); AA892813 (EST196616 cDNA); AA892986 (EST196789 cDNA); AA893082 (EST196885 cDNA); AA893185 (EST196988 cDNA); AA893199 (EST197002 cDNA); AA893224 (EST197027 cDNA); AA893320 (EST197123 cDNA); AA893584 (EST197387 cDNA); AA893690 (EST197493 cDNA); AA893708 (KIAA0560); AA893717 (EST197520 cDNA); AA893743 (EST197546 cDNA); AA893788 (ESTs, Highly similar to chromobox protein homolog 5); AA893946 (EST197749 cDNA); AA894305 (EST198108 cDNA); AA924925 (ER transmembrane protein Dri 42); AA942685 (cytosolic cysteine dioxygenase 1); AA955306 (ras-related protein rab10); AA955388 (Na^+K^+ transporting

ATPase 2, beta polypeptide 2); AA957132 (N-acetylglucosaminyltransferase I); AB000778 (Phoshpolipase D gene 1); AB006451 (Tim23); AB008538 (HB2); AB016532 (period homolog 2 (Drosophila)); AF000899 (p58/p45, nucleolin); AF007554 (Mucin1); AF007758 (synuclein, alpha); AF007890 (resection-induced TPI (rs11)); AF008554 (implantation-associated protein (IAG2)); AF013144 (MAP-kinase phosphatase (cpg21)); AF016269 (kallikrein 6 (neurosin, zyme)); AF016296 (neuropilin); AF019974 (Chromogranin B, parathyroid secretory protein); AF020046 (integrin alpha E1, epithelial-associated); AF021935 (Ser-Thr protein kinase); AF023087 (Early growth response 1); AF030050 (replication factor C); AF030088 (RuvB-like protein 1); AF040954 (putative protein phosphatase1 nuclear targeting subunit); AF051561 (solute carrier family 12, member 2); AF055477 (L-type voltage-dependent Ca^{2+} channel (?1D subunit)); AF074608 (MHC class I antigen (RT1.EC2) gene); AF076183 (cytosolic sorting protein PACS-1a (PACS-1)); AF095927 (protein phosphatase 2C); AI010110 (SH3-domain GRB2-like 1); AI012589 (glutathione S-transferase, pi 2); AI013627 (defender against cell death 1); AI013861 (3-hydroxyisobutyrate dehydrogenase); AI045249 (heat shock 70kD protein 8); AI102031 (myc box dependent interacting protein 1); AI102299 (Bid3); AI102839 (cerebellar Ca-binding protein, spot 35 protein); AI102868 (EST212157 cDNA); AI104388 (heat shock 27kD protein 1); AI136891 (zinc finger protein 36, C3H type-like 1); AI168942 (branched chain keto acid dehydrogenase E1); AI169265 (Atp6s1); AI171966 (ESTs, Highly similar to selenide, water dikinase 2); AI175973 (ESTs, Highly similar to NADH dehydrogenase); AI176491 (EST220076 cDNA); AI176595 (Cathepsin L); AI176621 (iron-responsive element-binding protein); AI177404 (EST221024 cDNA); AI178204 (EST221869 cDNA); AI178921 (Insulin degrading enzyme); AI228548 (ESTs, Highly similar to DKFZp586G0322.1); AI230247 (selenoprotein P, plasma, 1); AI230778 (ESTs, Highly similar to protein-tyrosine sulfotrans. 2); AI230914 (farnesyltransferase beta subunit); AI231807 (ferritin light chain 1); AI231807 (ferritin light chain 1); AI232268 (LDL receptor-related protein associated protein 1); AI235344 (geranylgeranyltransferase type I (GGTase-I)); AI237007 (ESTs, Highly similar to flavoprot.-ubiquin. Oxidoreduct.); AI638971 (mixed-tissue library cDNA clone rx04989 3); AI638997 (mixed-tissue library cDNA clone rx05048 3); AI639151 (mixed-tissue library cDNA clone rx02802 3); AI639209 (mixed-tissue library cDNA clone rx00680 3); AI639257 (mixed-tissue library cDNA clone rx01119 3); AI639477 (mixed-tissue library cDNA clone rx02351 3); D00569 (2,4-dienoyl CoA reductase 1,

mitochondrial); D10262 (choline kinase); D10699 (ubiquitin carboxy-terminal hydrolase L1); D10854 (aldehyde reductase); D10874 (lysosomal vacuolar proton pump (16 kDa)); D16478 (mitochondrial long-chain enoyl-CoA hydratase); D17309 (delta 4-3-ketosteroid-5-beta-reductase); D28110 (myelin-associated oligodendrocytic basic protein); D28110 (myelin-associated oligodendrocytic basic protein); D28557 (cold shock domain protein A); D29766 (v-crk-associated tyrosine kinase substrate); D37951 (MIBP1 (c-myc intron binding protein 1)); D45247 (proteasome subunit RCX); D45249 (protease (prosome, macropain) 28 subunit, alpha); D78308 (calreticulin); D83948 (adult liver S1-1 protein); D88586 (eosinophil cationic protein); D89340 (dipeptidylpeptidase III); D89730 (Fibulin 3, fibulin-like extracellular matrix protein 1); D90211 (Lysosomal-associated membrane protein 2); E03229 (cytosolic cysteine dioxygenase 1); H33086 (EST108750 cDNA); H33725 (associated molecule with the SH3 domain of STAM); J02752 (acyl-coA oxidase); J02773 (heart fatty acid binding protein); J05022 (peptidylarginine deiminase); J05031 (Isovaleryl Coenzyme A dehydrogenase); J05132 (UDP-glucuronosyltransferase); K02248 (Somatostatin); L00191 (Fibronectin 1); L13202 (RATHFH2 HNF-3/fork-head homolog-2 (HFH-2)); L19998 (sulfotransferase family 1A, phenol-preferring, member 1); L24896 (glutathione peroxidase 4); L25605 (Dynamin 2); L26292 (Kruppel-like factor 4 (gut)); L29573 (neurotransmitter transporter,noradrenalin); L42855 (transcription elongation factor B (SIII) polypeptide 2); M10068 (NADPH-cytochrome P-450 oxidoreductase); M13100 (long interspersed repetitive DNA sequence LINE3); M13100 (long interspersed repetitive DNA sequence LINE3); M19936 (Prosaposin- sphingolipid hydrolase activator); M23601 (Monoamine oxidase B); M24104 (synaptobrevin 2); M24104 (Vesicle-associated membrane protein (synaptobrevin 2)); M24852 (Neuron specific protein PEP-19 (Purkinje cell protein 4)); M31174 (thyroid hormone receptor alpha); M36453 (Inhibin, alpha); M55015 (nucleolin); M57276 (Leukocyte antigen (Ox-44)); M58404 (thymosin, beta 10); M80550 (adenylyl cyclase); M83745 (Protein convertase subtilisin / kexin, type I); M89646 (ribosomal protein S24); M91234 (VL30 element); M93273 (somatostatin receptor subtype 2); M93669 (Secretogranin II); M99485 (Myelin oligodendrocyte glycoprotein); S53527 (S100 calcium-binding protein, beta (neural)); S61868 (Ryudocan/syndecan 4); S72594 (tissue inhibitor of metalloproteinase 2); S77492 (Bone morphogenetic protein 3); S77858 (non-muscle myosin alkali light chain); U04738 (Somatostatin receptor subtype 4); U07619 (Coagulation factor III (thromboplastin, tissue factor)); U08259 (Glutamate receptor, N-methyl D-aspartate 2C); U10357 (pyruvate

dehydrogenase kinase 2 subunit p45 (PDK2)); U14950 (tumor suppressor homolog (synapse associ. protein)); U17254 (immediate early gene transcription factor NGFI-B); U17254 (immediate early gene transcription factor NGFI-B); U18771 (Ras-related protein Rab-26); U27518 (UDP-glucuronosyltransferase); U28938 (receptor-type protein tyrosine phosphatase D30); U38379 (Gamma-glutamyl hydrolase); U38801 (DNA polymerase beta); U67080 (r-MyT13); U67136 (A kinase (PRKA) anchor protein 5); U67137 (guanylate kinase associated protein); U72620 (Lot1); U75405 (procollagen, type I, alpha 1); U75917 (clathrin-associated protein 17); U77777 (interleukin 18); U78517 (cAMP-regulated guanine nucleotide exchange factor II); U89905 (alpha-methylacyl-CoA racemase); V01244 (Prolactin); X02904 (glutathione S-transferase P subunit); X05472 (2.4 kb repeat DNA right terminal region); X06769 (FBJ v-fos oncogene homolog); X06916 (S100 calcium-binding protein A4); X13905 (ras-related rab1B protein); X14323 (Fc receptor, IgG, alpha chain transporter); X16933 (RNA binding protein p45AUF1); X53427 (glycogen synthase kinase 3 alpha (EC 2.7.1.37)); X53504 (ribosomal protein L12); X54467 (cathepsin D); X55153 (ribosomal protein P2); X57281 (Glycine receptor alpha 2 subunit); X58294 (carbonic anhydrase 2); X59737 (ubiquitous mitochondrial creatine kinase); X60212 (ASI homolog of bacterial ribosomal subunit protein L22); X62950 (pBUS30 with repetitive elements); X67805 (Synaptonemal complex protein 1); X72757 (cox VIa gene (liver)); X74226 (LL5 protein); X76489 (CD9 cell surface glycoprotein); X76985 (latexin); X82445 (nuclear distribution gene C homolog (*Aspergillus*)); X84039 (lumican); X89696 (TPCR06 protein); X97443 (integral membrane protein Tmp21-I (p23)); X98399 (solute carrier family 14, member 1); Y17295 (thiol-specific antioxidant protein (1-Cys peroxiredoxin)); Z48225 (protein synthesis initiation factor eIF-2B delta subunit); Z49858 (plasmolipin)

[107] Using the method of the invention, we have also identified a set of genes and ESTs that changed with age ($p \leq .05$), but which are correlated with cognitive performance in behavioral tests. These include L03294 (Lpl, lipoprotein lipase); M18416 (Egr1, Early growth response 1 (Krox-24)); S68245 (Ca4, carbonic anhydrase 4); M64780 (Agrn, Agrin); M27207 (Col1a1, Procollagen- type I (alpha 1)); X16554 (Prps1, Phosphoribosyl pyrophosphate synthetase 1); M92433 (NGFI-C, Zinc-finger transcription factor (early response gene)); AA859975 (LOC64201, 2-oxoglutarate carrier); L08595 (Nuclear receptor subfamily 4, group A, member 2); M24542 (RISP, Rieske iron-sulfur protein); AI030089 (Nopp130, nucleolar phosphoprotein p130); AF104362 (Omd, Osteomodulin (osteoadherin));

L46873 (Slc15a1, Oligopeptide transporter); AI176689 (MAPKK 6, mitogen-activated protein kinase kinase 6); U66470 (rCGR11, Cell growth regulator); AF016387 (RXRG, retinoid X-receptor gamma); M18467 (Got2, glutamate oxaloacetate transaminase 2); X54793 (Hsp60, heat shock protein 60); X64401 (Cyp3a3, Cytochrome P450- subfamily IIIA (polypeptide 3)); M37584 (H2afz, H2A histone family (member Z)); L21192,(GAP-43, membrane attached signal protein 2 (brain)); AA875047 (TCPZ, T-complex protein 1 (zeta subunit)); U90610 (Cxcr4, CXC chemokine receptor); AF003904 (CRH-binding protein); U83880 (GPDH-M, glycerol-3-phosphate dehydrogenase, mitochondrial); X89703 (TPCR19, Testis Polymerase Chain Reaction product 19); D63886 (MMP16, matrix metalloproteinase 16); J05499 (GLS, glutaminase (mitochondrial)); D21799 (Psmb2, Proteasome subunit (beta type 2)); AA800794 (HT2A, zinc-finger protein); U90887 (Arg2, arginase type II); S82649 (Narp, neuronal activity-regulated pentraxin); M74223 (VGF, neurosecretory protein); AA874794 (Bex3, brain expressed X-linked 3); M15191 (Tac1, Tachykinin); AA892506 (coronin, actin binding protein 1A); L04485 (MAPPK1, mitogen-activated protein kinase kinase 1); AA799641 (S164, Contains a PWI domain associated with RNA splicing); AA817892 (Gnb2, Guanine nucleotide binding protein (beta 2 subunit)); AA893939 (DSS1, deleted in split hand/ split foot protein 1); AF000901 (P58/P45, Nucleoporin p58); AF087037 (Btg3, B-cell translocation gene 3); AB000280 (PHT1, peptide/histidine transporter); M87854 (Beta-ARK-1, beta adrenergic receptor kinase 1); U06099 (Prdx2, Peroxiredoxin 2); AF058795 (Gb2, GABA-B receptor); AA800517 (VAP1, vesicle associated protein); U63740 (Fez1, Protein kinase C-binding protein Zeta1); U53922 (Hsj2, DnaJ-like protein (RDJ1)); U78102 (Egr2, Early growth response 2); U44948 (SmLIM, smooth muscle cell LIM protein); U87627 (MCT3, putative monocarboxylate transporter); AB020504 (PMF31, highly homologus to mouse F-box-WD40 repeat protein 6); M21354 (Col3a1, collagen type III alpha-1); AA893664 (Temo, sertoli cell marker (KIAA0077 protein fragment)); AB010437 (CDH8, Cadherin-8); M22756 (Ndufv2, mitochondrial NADH dehydrogenase (24 kDa)); AA799389 (Rab3B, ras-related protein); AI172476 (Tieg-1, TGF-beta-inducible early growth response protein 1); AF091563 (Olfactory receptor); M64376 (Olfactory protein); J04488 (Ptgds, Prostaglandin D synthase); X71127 (c1qb, complement component 1- q (beta polypeptide)); J03752 (Microsomal GST-1, glutathione S-transferase); J03481 (Qdpr, Dihydropteridine reductase); L40362 (MHC class I RT1.C-type protein); M94918 (Hbb, beta hemoglobin); M55534 (Cryab, alpha crystallin polypeptide 2); U17919

(Aif1, allograft inflammatory factor 1); M15562 (MHC class II RT1.u-D-alpha chain); AA799645 (Phospholemman, FXYD domain-containing ion transport regulator 1); X13044 (Cd74, CD74 antigen); M24324 (RTS, MHC class I RT1 (RTS) (u haplotype)); U31866 (Nclone10); M32062 (Fcgr3, Fc IgG receptor III (low affinity)); AF095741 (Mg87); L03201 (Ctss, cathepsin S); M27905 (Rpl21, Ribosomal protein L21); D38380 (Tf, Transferrin); AA893493 (RPL26, Ribosomal protein L26); AJ222813 (Il18, interleukin 18); E13541 (Cspg5, chondroitin sulfate proteoglycan 5); X54096 (Lcat, Lecithin-cholesterol acyltransferase); L40364 (RT1Aw2, RT1 class Ib); D28111 (MOBP, myelin-associated oligodendrocytic basic protein); M32016 (Lamp2, lysosomal-associated membrane protein 2); X13167 (NF1-A, nuclear factor 1 A); U26356 (S100A1, S100 protein (alpha chain)); AI231213 (Kangai 1, suppression of tumorigenicity 6); AI170268 (Ptgfr, Prostaglandin F receptor); X62952 (Vim, vimentin); AI014169 (Vdup1, vitamin D-upregulated); AA850219 (Anx3, Annexin A3); D84477 (Rhoa, ras-related homolog A2); X52477 (C3, Complement component 3); X52619 (Rpl28, Ribosomal protein L28); X06554 (S-MAG, myelin-associated glycoprotein C-term); Z50144 (Kat2, kynurenine aminotransferase II); X14181 (RPL18A, Ribosomal protein L18a); AA892333 (Tuba1, alpha-tubulin); U67082 (KZF-1, Kruppel associated box (KRAB) zinc finger 1); U11760 (Vcp, valosin-containing protein); AF048828 (VDAC1, voltage-dependent anion channel 1); M31076 (TNF-alpha, Transforming growth factor (alpha)); S83279 (HSDIV, 17-beta-hydroxysteroid dehydrogenase type IV); AI102103 (Pik4cb, Phosphatidylinositol 4-kinase); X56325 (Hba1, alpha 1 hemoglobin); X73371 (FCGR2, Low affinity immunoglobulin gamma Fc receptor II); X78848 (Gsta1, Glutathione-S-transferase (alpha type)); U92564 (Roaz, Olf-1/EBF associated Zn finger protein); AI171462 (Cd24, CD24 antigen); X83231 (PAIHC3, Pre-alpha-inhibitor, heavy chain 3); AF097593 (Ca4, cadherin 2- type 1 (neuronal)); X68283 (Rpl29, Ribosomal protein L29); S55427 (Pmp, peripheral myelin protein); AA818025 (Cd59, CD59 antigen); E01534 (Rps15, Ribosomal protein S15); U37138 (Sts, Steroid sulfatase); X55572 (Apod, Apolipoprotein D); AI028975 (AP-1, adaptor protein complex (beta 1)); L16995 (ADD1, adipocyte determination/ differentiation-dependent factor 1); U07971 (Transamidinase, Glycine amidinotransferase, mitochondrial); L07736 (Cpt1a, Carnitine palmitoyltransferase 1 alpha (liver)); AI237535 (LitaF, LPS-induced TNF-alpha factor); AI175486 (Rps7, Ribosomal protein S7); U32498 (RSEC8, rat homolog of yeast sec8); X53504 (RPL12, Ribosomal protein L12); AF023621 (Sort1, sortilin); AF083269

(P41-Arc, actin-related protein complex 1b); AA891810 (GST, Glutathione S-transferase); M77694 (Fah, fumarylacetoacetate hydrolase); M22357 (MAG, myelin-associated glycoprotein); AI230712 (Pace4, Subtilisin - like endoprotease); AF008439 (NRAMP2, Natural resistance-associated macrophage protein 2); U77829 (Gas-5, growth arrest homolog); U92081 (Gp38, Glycoprotein 38); AA891445 (Skd3, suppressor of K^+ transport defect 3); AI177161 (Nfe2l2, NF-E2-related factor 2); AF031430 (Stx7, Syntaxin 7); L35921 (Ggamma, GTP-binding protein (gamma subunit)); X62322 (Grn, Granulin); AF028784 (GFAP, glial fibrillary acidic protein); and AI234146 (Csrp1, Cysteine rich protein 1).

[108] Using the method of the invention, we have further identified a set of genes and ESTs that changed with age ($p \leq .01$). These include AA891651 (rc_AA891651 EST195454 cDNA); AI070108 (rc_AI070108 UI-R-Y0-lu-a-09-0-UI.s1 cDNA); AI176689 (mitogen-activated protein kinase kinase 6); AI012051 (rc_AI012051 EST206502 cDNA); AI233365 (rc_AI233365 EST230053 cDNA); AA892532 (rc_AA892532 EST196335 cDNA); AA893185 (rc_AA893185 EST196988 cDNA); AA964320 (rc_AA964320 UI-R-C0-gu-e-09-0-UI.s1 cDNA); AA963449 (rc_AA963449 UI-R-E1-gj-e-08-0-UI.s1 cDNA); AA859632 (rc_AA859632 UI-R-E0-bs-h-08-0-UI.s1 cDNA); AI169265 (Atp6s1); AA850781 (rc_AA850781 EST193549 cDNA); AJ222813 (interleukin 18); D38380 (Transferrin); J03481 (dihydropteridine reductase); M24542 (Rieske iron-sulfur protein); L03294 (Lipoprotein lipase); L19998 (sulfotransferase family 1A, phenol-preferring, member 1); U53922 (DnaJ-like protein (RDJ1)); X54793 (liver heat shock protein (hsp60)); X62952 (vimentin); M55534 (Crystallin, alpha polypeptide 2); J03752 (microsomal glutathione S-transferase 1); X64401 (Cytochrome P450, subfamily IIIA, polypeptide 3); X78848 (Gsta1); AF016387 (retinoid X receptor gamma); AF031430 (syntaxin 7); AF051561 (solute carrier family 12, member 2); AF076183 (cytosolic sorting protein PACS-1a (PACS-1)); AF095576 (adaptor protein with pleckstrin homology and src homology 2 domains); AF095741 (MG87); AF097593 (cadherin 2, type 1, N-cadherin (neuronal)); AF104362 (osteoadherin); D10699 (ubiquitin carboxy-terminal hydrolase L1); D28111 (myelin-associated oligodendrocytic basic protein); D37951 (MIBP1 (c-myc intron binding protein 1)); D84477 (RhoA); L13202 (RATHFH2 HNF-3/fork-head homolog-2 (HFH-2)); L26292 (Kruppel-like factor 4 (gut)); L46873 (solute carrier family 15 (oligopeptide transporter), member 1); M13100 (RATLIN3A long interspersed repetitive DNA sequence LINE3 (L1Rn)); M27207 (procollagen, type I, alpha 1); M92433 (Zinc-finger transcription factor NGFI-C (early

response gene)); M94918 (Hemoglobin, beta); M94919 (Hemoglobin, beta); S55427 (Peripheral myelin protein); S68245 (carbonic anhydrase 4); S82649 (Narp=neuronal activity-regulated pentraxin); U10894 (allograft inflammatory factor 1); U26356 (RNSHUNA1 S100A1 gene); U75397 (RNKROX2 Krox-24); U75405 (procollagen, type I, alpha 1); U77829 (RNU77829 gas-5 growth arrest homolog non-translated sequence); U92081 (glycoprotein 38); X06554 (RNMAGSR myelin-associated glycoprotein (S-MAG) C-term); X13167 (Nuclear Factor IA); X14181 (RRRPL18A ribosomal protein L18a); X56325 (Hemoglobin, alpha 1); X60351 (Crystallin, alpha polypeptide 2); E13541 (chondroitin sulfate proteoglycan 5); M22357 (1B236/myelin-associated glycoprotein (MAG)); M24026 (RT1 class Ib gene); M24324 (MHC class I RT1 (RTS) (u haplotype)); J04488 (Prostaglandin D synthase); M15191 (Tachykinin (substance P, neurokinin A, neuropeptide K, neuropeptide gamma)); M74223 (VGF); U17254 (immediate early gene transcription factor NGFI-B); U08259 (Glutamate receptor, ionotropic, N-methyl D-aspartate 2C); U19866 (activity regulated cytoskeletal-associated protein); L40364 (RT1 class Ib gene); U17919 (allograft inflammatory factor 1); U78102 (early growth response 2); U67082 (KRAB-zinc finger protein KZF-1); U77777 (interleukin 18); D78018 (Nuclear Factor IA); U92564 (Olf-1/EBF associated Zn finger protein Roaz); AF008439 (Solute carrier family 11 member 2 (natural resistance-associated macrophage protein 2)); AB003726 (RuvB-like protein 1); M83561 (Glutamate receptor, ionotropic, kainate 1); AI639151 (mixed-tissue library cDNA clone rx02802 3); AI639247 (mixed-tissue library cDNA clone rx03939 3); AI639381 (mixed-tissue library cDNA clone rx01495 3); AI639532 (mixed-tissue library cDNA clone rx01030 3); AA799645 (FXYD domain-containing ion transport regulator 1); AA900516 (Pdi2); AI014169 (Vdup1); AI030089 (Nopp140); AI102299 (Bid3); AA818025 (CD59 antigen); AI170268 (Prostaglandin F receptor); AI171462 (CD24 antigen); AI171966 (ESTs, Highly similar to SPS2 MOUSE SELENIDE,WATER DIKINASE 2 [M.musculus]); AI176456 (ESTs, Weakly similar to ABP2_HUMAN ENDOTHELIAL ACTIN-BINDING PROTEIN [H.sapiens]); AI177161 (NF-E2-related factor 2); AI179576 (Hemoglobin, beta); AI230712 (Subtilisin - like endoprotease); AI230914 (farnesyltransferase beta subunit); AI231213 (kangai 1 (suppression of tumorigenicity 6), prostate); AI237731 (Lipoprotein lipase); M83745 (Protein convertase subtilisin / kexin, type I); M27905 (ribosomal protein L21); M32016 (Lysosomal-associated membrane protein 2); M11071 (RT1 class Ib gene); M15562 (MHC class II RT1.u-D-alpha chain); M15880 (Neuropeptide Y); L08595 (nuclear

receptor subfamily 4, group A, member 2); M18416 (Early growth response 1); L40362 (MHC class I RT1.C-type protein); Z50144 (kynurenine/alpha-aminoadipate aminotransferase); X71127 (complement component 1, q subcomponent, beta polypeptide); U44948 (smooth muscle cell LIM protein (SmLIM)); AA850219 (Annexin A3); X73371 (FCGR2); X57281 (Glycine receptor alpha 2 subunit (glycine receptor, neonatal)); X83231 (pre-alpha-inhibitor); X52477 (Complement component 3); X16554 (phosphoribosyl pyrophosphate synthetase 1); X78605 ((Sprague Dawley) rab4b ras-homologous GTPase); X82445 (nuclear distribution gene C homolog (Aspergillus)); X52619 (ribosomal protein L28); X68283 (ribosomal protein L29); X13044 (CD74 antigen (invariant polpypeptide of major histocompatibility class II antigen-associated)); X54096 (Lecithin-cholesterol acyltransferase); U31866 (Nclone10); U72620 (Lot1); U66470 (rCGR11); M31018 (RT1 class Ib gene); U90887 (arginase type II); M18467 (Glutamate oxaloacetate transaminase 2, mitochondrial (aspartate aminotransferase 2)); M64780 (Agrin); U87627 (putative monocarboxylate transporter (MCT3)); AF019974 (Chromogranin B, parathyroid secretory protein); L03201 (cathepsin S); AB008538 (HB2); D89340 (dipeptidylpeptidase III); M77694 (fumarylacetoacetate hydrolase); M32062 (Fc-gamma receptor); L21192 (brain abundant, membrane attached signal protein 2); M37584 (H2afz); AA858588 (ESTs, Weakly similar to ODP2 RAT DIHYDROLIPOAMIDE ACETYLTRANSFERASE COMPONENT OF PYRUVATE DEHYDROGENASE COMPLEX [R.norvegicus]); AA858617 (rc_AA858617 UI-R-E0-bq-b-06-0-UI.s1 cDNA); AA859562 (rc_AA859562 UI-R-E0-bv-b-03-0-UI.s1 cDNA); AA859626 (rc_AA859626 UI-R-E0-bs-h-02-0-UI.s1 cDNA); AA859690 (rc_AA859690 UI-R-E0-bx-e-11-0-UI.s1 cDNA); AA859777 (rc_AA859777 UI-R-E0-bu-e-10-0-UI.s1 cDNA); AA859975 (LOC64201); AA860030 (UI-R-E0-bz-e-07-0-UI.s2 cDNA); AA866291 (rc_AA866291 UI-R-A0-ac-e-12-0-UI.s3 cDNA); AA866409 (rc_AA866409 UI-R-E0-ch-a-03-0-UI.s1 cDNA); AA866411 (NDN); AA874794 (Bex3); A874887 (rc_AA874887 UI-R-E0-ci-g-10-0-UI.s1 cDNA); AA875004 (rc_AA875004 UI-R-E0-cb-b-07-0-UI.s1 cDNA); AA875037 (rc_AA875037 UI-R-E0-cb-a-03-0-UI.s1 cDNA); AA875047 (TCPZ); AA875059 (rc_AA875059 UI-R-E0-cb-f-04-0-UI.s1 cDNA); AA875129 (rc_AA875129 UI-R-E0-bu-e-01-0-UI.s2 cDNA); H31418 (rc_H31418 EST105434 cDNA); H31665 (rc_H31665 EST105952 cDNA); H32977 (rc_H32977 EST108553 cDNA); H33725 (associated molecule with the SH3 domain of STAM); AA891037 (rc_AA891037 EST194840 cDNA); AA891445 (Skd3); AA891690 (ESTs, Weakly similar to

SERC_HUMAN PHOSPHOSERINE AMINOTRANSFERASE [H.sapiens]); AA891717 (USF1); AA891734 (rc_AA891734 EST195537 cDNA); AA891785 (rc_AA891785 EST195588 cDNA); AA891810 (ESTs, Highly similar to GTK1 RAT GLUTATHIONE S-TRANSFERASE, MITOCHONDRIAL [R.norvegicus]); AA891965 (rc_AA891965 EST195768 cDNA); AA892333 (Tuba1); AA892353 (ESTs, Moderately similar to JC5823 NADH dehydrogenase [H.sapiens]); AA892511 (rc_AA892511 EST196314 cDNA); AA892986 (rc_AA892986 EST196789 cDNA); AA893032 (ESTs, Moderately similar to CALX RAT CALNEXIN PRECURSOR [R.norvegicus]); AA893082 (rc_AA893082 EST196885 cDNA); AA893493 (RPL26); AA893607 (rc_AA893607 EST197410 cDNA); AA893708 (KIAA0560); AA893743 (rc_AA893743 EST197546 cDNA); AA894104 (rc_AA894104 EST197907 cDNA); AA799449 (EST, Weakly similar to UBP4 MOUSE UBIQUITIN CARBOXYL-TERMINAL HYDROLASE 4 [M.musculus]); AA799779 (acyl-CoA:dihydroxyacetonephosphate acyltransferase); AA799803 (ESTs, Weakly similar to K1CU RAT KERATIN, TYPE I CYTOSKELETAL 21 [R.norvegicus]); AA799854 (rc_AA799854 EST189351 cDNA); AA799996 (rc_AA799996 EST189493 cDNA); AA800693 (rc_AA800693 EST190190 cDNA); AA800708 (ESTs, Weakly similar to S28312 hypothetical protein F02A9.4 -Caenorhabditis elegans [C.elegans]); AA800794 (HT2A); and AA800948 (Tuba4).

[109] We have also identified age-related ESTs, including AA963449 (UI-R-E1-gj-e-08-0-UI.s1 cDNA); AA892532 (EST196335 cDNA); AA859626 (UI-R-E0-bs-h-02-0-UI.s1 cDNA); AA893743 (EST197546 cDNA); AI233365 (EST230053 cDNA); H31665 (EST105952 cDNA); AA892353 (ESTs, Moderately similar to JC5823 NADH dehydrogenase); AI639247 (mixed-tissue library cDNA clone rx03939 3); AA858617 (UI-R-E0-bq-b-06-0-UI.s1 cDNA); AI639429 (mixed-tissue library cDNA clone rx00973 3); AA858620 (UI-R-E0-bq-b-09-0-UI.s1 cDNA); AA866291 (UI-R-A0-ac-e-12-0-UI.s3 cDNA); AA894104 (EST197907 cDNA); AA799996 (EST189493 cDNA); AA892805 (EST196608 cDNA); AI639019 (mixed-tissue library cDNA clone rx01107 3); AA799538 (EST189035 cDNA); AI070108 (UI-R-Y0-lu-a-09-0-UI.s1 cDNA); AA866409 (UI-R-E0-ch-a-03-0-UI.s1 cDNA); AA859632 (UI-R-E0-bs-h-08-0-UI.s1 cDNA); AA891651 (EST195454 cDNA); AA893032 (ESTs, Moderately similar to CALX calnexin precursor); AA891965 (EST195768 cDNA); AA800708 (ESTs, Weakly similar to S28312 hypothetical protein F02A9.4); AA964320 (UI-R-C0-gu-e-09-0-UI.s1 cDNA); AA893173 (EST196976

cDNA); H32977 (EST108553 cDNA); AA874887 (UI-R-E0-ci-g-10-0-UI.s1 cDNA); AA850781 (EST193549 cDNA); AI176456 (ESTs, Weakly similar to endothelial actin-binding protein); H31418 (EST105434 cDNA); AA858588 (ESTs, Weakly similar to ODP2 dihydrolipoamide acetyl transferase); AA891785 (EST195588 cDNA); AA799803 (ESTs, Weakly similar to K1CU cytoskeletal keratin (type 1)); AA799449 (EST, Weakly similar to UBP4 ubiquitin carboxyl-terminal hydrolase 4); AA859777 (UI-R-E0-bu-e-10-0-UI.s1 cDNA); AI639532 (mixed-tissue library cDNA clone rx01030 3); AA875059 (UI-R-E0-cb-f-04-0-UI.s1 cDNA); AI012051 (EST206502 cDNA); AA800549 (EST190046 cDNA); AA799854 (EST189351 cDNA); and AA892520 (EST196323 cDNA).

[110] Those of skill in the genomics art will understand that the identified genes and ESTs have utility as biomarkers of brain aging. Those of skill in the genomics art will understand that the mammalian homologues (including rat, mouse and human homologues) the identified genes and ESTs are also as biomarkers of brain aging. The easiest method for identifying mammalian homologues of the identified genes and ESTs is by identifying the homologues in the GenBank database, preferably, or in the SwissProtein and the Genome Ontology databases. Additional guidance as to homology can be obtained by using commercially available computer programs, such as DNA Strider and Wisconsin GCG, and following the instructions for the determination of the degree of homolgy between selected polynucleotides.

[111] The foregoing description has been presented only for the purposes of illustration and is not intended to limit the invention to the precise form disclosed, but by the claims appended hereto.

CLAIMS

What is claimed is:

1. A method for identifying a biomarker for brain aging, wherein the biomarker is a polynucleotide or a polypeptide encoded by said polynucleotide, comprising the steps of:

(a) obtaining a set of polynucleotides obtained from a set of brain samples, wherein the members of the set of brain samples were obtained from members of a set of mammals, wherein the set of mammals contains more than two members and wherein the set of mammals comprises young, mid-aged and aged members;

(b) identifying the identity and amount of the members of the set of polynucleotides present in the brain samples;

(c) deleting from the set of polynucleotides;

(1) quality control oligonucleotides;

(2) polynucleotides in which the difference between the young and the aged members did not comprise at least 75% of the maximum normalized difference among the members; and

(d) testing by a conventional statistical test for a significant effect of aging across the young, mid-aged and aged members;

wherein the polynucleotides, and polypeptide encoded by said polynucleotides, that are both significantly altered in an age-dependent fashion across age are identified as biomarkers for brain aging.

2. The identification method of claim 1, further comprising the step of:

(e) correlating the identity and amount of the members of the set of polynucleotides present in the brain samples with cognitive performance in a behavioral test, using a conventional statistical correlation test;

wherein the polynucleotides, and polypeptide encoded by said polynucleotides, that are both significantly altered in an age-dependent fashion as well as significantly correlated with cognitive performance across age are identified as biomarkers for brain aging.

3. The identification method of claim 1, wherein the biomarker for brain aging is a biomarker for an age-related neurodegenerative condition.

4. The identification method of claim 3, wherein the age-related neurodegenerative condition is Alzheimer's disease or Parkinson's disease.

5. The identification method of claim 1, wherein the brain sample is a hippocampal sample.

6. The identification method of claim 1, wherein the mammal is selected from the group consisting of rat, mouse and human.

7. The identification method of claim 1, wherein the biomarker for brain aging is an expressed sequence tag (EST).

8. The identification method of claim 1, further comprising, in the deletion step (c), the step of:

(3) deleting, from the set of polynucleotides, polynucleotides for expressed sequence tags (ESTs) which have not been associated with known genes.

9. The identification method of claim 1, further comprising, in the deletion step (c), the step of:

(3) deleting, from the set of polynucleotides, polynucleotides that are not expressed sequence tags (ESTs).

10. The identification method of claim 1, wherein the conventional statistical test in step (d) is ANOVA or student's t test.

11. The identification method of claim 1, wherein the testing in step (d) is testing by 1-way ANOVA for a significant effect of aging $p < 0.05$.

12. The identification method of claim 1, wherein the behavioral tests of step (e) specifically test for cognitive deficits related to the region of the brain from which the brain sample has been obtained in step (a).

13. The identification method of claim 1, wherein the identification of the identity and amount of the members of the set of polynucleotides present in the brain samples in step (b) is by microarray analysis.

14. The identification method of claim 1, wherein the significant effect in step (d) is $p < 0.025$.

15. The identification method of claim 1, wherein the significant effect in step (d) is $p < 0.01$.

16. The identification method of claim 1, wherein the significant effect in step (d) is $p < 0.001$.

17. The identification method of claim 1, wherein the behavioral test in step (e) is selected from the group consisting of the Morris spatial water maze (SWM) and the object memory task (OMT).

18. The identification method of claim 1, wherein the behavioral tests in step (e) are selected from the group consisting of tests for Alzheimer's disease and tests for Parkinson's disease.

19. The identification method of claim 1, wherein the correlation of the identity and amount of the members of the set of polynucleotides present in the brain samples with cognitive performance in behavioral tests is a Pearson or Spearman correlation of expression with behavior.

20. The identification method of claim 19, wherein the correlation is $p < 0.025$.

21. The identification method of claim 19, wherein the correlation is $p < 0.01$.

22. The identification method of claim 19, wherein the correlation is $p < 0.001$.

23. A set of biomarkers for brain aging, comprising mammalian polynucleotides and polypeptides encoded by said polynucleotides:
    (a) wherein the set of biomarkers comprises at least two members;
    (b) wherein the brain expression patterns of the members of the set are significantly altered with aging as determined by a conventional statistical test, with $p < 0.05$;
    (c) wherein the brain expression patterns of the members of the set are correlated across age groups with cognitive performance in behavioral tests, using a conventional statistical correlation test with a correlation of $p < 0.05$ between brain expression and cognitive performance; and
    (d) wherein the cognitive performance in behavioral tests significantly altered with aging as determined by a conventional statistical test.

24. The set of biomarkers of claim 23, wherein the biomarkers further correlate with a behavioral measure of functional impairment.

25. The set of biomarkers of claim 23, wherein the behavioral measure of functional impairment is a test for an age-related neurodegenerative condition.

26. The set of biomarkers of claim 25, wherein the age-related neurodegenerative condition is Alzheimer's disease or Parkinson's disease.

27. The set of biomarkers of claim 23, wherein the mammal is selected from the group consisting of rat, mouse and human.

28. The set of biomarkers of claim 23, wherein the conventional statistical method in step (b) is ANOVA or student's t-test.

29. The set of biomarkers of claim 23, wherein the correlation in step (c) is tested by a correlation test selected from the group consisting of Pearson's correlation test and Spearman's correlation test.

30. The set of biomarkers of claim 23, wherein the age groups in step (c) comprises young, mid-aged and aged.

31. The set of biomarkers of claim 23, wherein the conventional statistical test in step (d) is ANOVA or student's t-test.

32. The set of biomarkers of claim 23, wherein at least one member of the set of biomarkers is a polynucleotide, or a polypeptide encoded by said polynucleotide, selected from the group consisting of L03294 (Lpl, lipoprotein lipase); M18416 (Egr1, Early growth response 1 (Krox-24)); S68245 (Ca4, carbonic anhydrase 4); M64780 (Agrn, Agrin); M27207 (Col1a1, Procollagen- type I (alpha 1)); X16554 (Prps1, Phosphoribosyl pyrophosphate synthetase 1); M92433 (NGFI-C, Zinc-finger transcription factor (early response gene)); AA859975 (LOC64201, 2-oxoglutarate carrier); L08595 (Nuclear receptor subfamily 4, group A, member 2); M24542 (RISP, Rieske iron-sulfur protein); AI030089 (Nopp130, nucleolar phosphoprotein p130); AF104362 (Omd, Osteomodulin (osteoadherin)); L46873 (Slc15a1, Oligopeptide transporter); AI176689 (MAPKK 6, mitogen-activated protein kinase kinase 6); U66470 (rCGR11, Cell growth regulator); AF016387 (RXRG, retinoid X-receptor gamma); M18467 (Got2, glutamate oxaloacetate transaminase 2); X54793 (Hsp60, heat shock protein 60); X64401 (Cyp3a3, Cytochrome P450- subfamily IIIA (polypeptide 3)); M37584 (H2afz, H2A histone family (member Z)); L21192 (GAP-43, membrane attached signal protein 2 (brain)); AA875047 (TCPZ, T-complex protein 1 (zeta subunit)); U90610 (Cxcr4, CXC chemokine receptor); AF003904 (CRH-binding protein); U83880 (GPDH-M, glycerol-3-phosphate dehydrogenase, mitochondrial); X89703 (TPCR19, Testis Polymerase Chain Reaction product 19);

D63886 (MMP16, matrix metalloproteinase 16); J05499 (GLS, glutaminase (mitochondrial)); D21799 (Psmb2, Proteasome subunit (beta type 2)); AA800794 (HT2A, zinc-finger protein); U90887 (Arg2, arginase type II); S82649 (Narp, neuronal activity-regulated pentraxin); M74223 (VGF, neurosecretory protein); AA874794 (Bex3, brain expressed X-linked 3); M15191 (Tac1, Tachykinin); AA892506 (coronin, actin binding protein 1A); L04485 (MAPPK1, mitogen-activated protein kinase kinase 1); AA799641 (S164, Contains a PWI domain associated with RNA splicing); AA817892 (Gnb2, Guanine nucleotide binding protein (beta 2 subunit)); AA893939 (DSS1, deleted in split hand/ split foot protein 1); AF000901 (P58/P45, Nucleoporin p58); AF087037 (Btg3, B-cell translocation gene 3); AB000280 (PHT1, peptide/histidine transporter); M87854 (Beta-ARK-1, beta adrenergic receptor kinase 1); U06099 (Prdx2, Peroxiredoxin 2); AF058795 (Gb2, GABA-B receptor); AA800517 (VAP1, vesicle associated protein); U63740 (Fez1, Protein kinase C-binding protein Zeta1); U53922 (Hsj2, DnaJ-like protein (RDJ1)); U78102 (Egr2, Early growth response 2); U44948 (SmLIM, smooth muscle cell LIM protein); U87627 (MCT3, putative monocarboxylate transporter); AB020504 (PMF31, highly homologus to mouse F-box-WD40 repeat protein 6); M21354 (Col3a1, collagen type III alpha-1); AA893664 (Temo, sertoli cell marker (KIAA0077 protein fragment)); AB010437 (CDH8, Cadherin-8); M22756 (Ndufv2, mitochondrial NADH dehydrogenase (24 kDa)); AA799389 (Rab3B, ras-related protein); AI172476 (Tieg-1, TGF-beta-inducible early growth response protein 1); AF091563 (Olfactory receptor); M64376 (Olfactory protein); J04488 (Ptgds, Prostaglandin D synthase); X71127 (c1qb, complement component 1- q (beta polypeptide)); J03752 (Microsomal GST-1, glutathione S-transferase); J03481 (Qdpr, Dihydropteridine reductase); L40362 (MHC class I RT1.C-type protein); M94918 (Hbb, beta hemoglobin); M55534 (Cryab, alpha crystallin polypeptide 2); U17919 (Aif1, allograft inflammatory factor 1); M15562 (MHC class II RT1.u-D-alpha chain); AA799645 (Phospholemman, FXYD domain-containing ion transport regulator 1); X13044 (Cd74, CD74 antigen); M24324 (RTS, MHC class I RT1 (RTS) (u haplotype)); U31866 (Nclone10); M32062 (Fcgr3, Fc IgG receptor III (low affinity)); AF095741 (Mg87); L03201 (Ctss, cathepsin S); M27905 (Rpl21, Ribosomal protein L21); D38380 (Tf, Transferrin); AA893493 (RPL26, Ribosomal

protein L26); AJ222813 (Il18, interleukin 18); E13541 (Cspg5, chondroitin sulfate proteoglycan 5); X54096 (Lcat, Lecithin-cholesterol acyltransferase); L40364 (RT1Aw2, RT1 class Ib); D28111 (MOBP, myelin-associated oligodendrocytic basic protein); M32016 (Lamp2, lysosomal-associated membrane protein 2); X13167 (NF1-A, nuclear factor 1 A); U26356 (S100A1, S100 protein (alpha chain)); AI231213 (Kangai 1, suppression of tumorigenicity 6); AI170268 (Ptgfr, Prostaglandin F receptor); X62952 (Vim, vimentin); AI014169 (Vdup1, vitamin D-upregulated); AA850219 (Anx3, Annexin A3); D84477 (Rhoa, ras-related homolog A2); X52477 (C3, Complement component 3); X52619 (Rpl28, Ribosomal protein L28); X06554 (S-MAG, myelin-associated glycoprotein C-term); Z50144 (Kat2, kynurenine aminotransferase II); X14181 (RPL18A, Ribosomal protein L18a); AA892333 (Tuba1, alpha-tubulin); U67082 (KZF-1, Kruppel associated box (KRAB) zinc finger 1); U11760 (Vcp, valosin-containing protein); AF048828 (VDAC1, voltage-dependent anion channel 1); M31076 (TNF-alpha, Transforming growth factor (alpha)); S83279 (HSDIV, 17-beta-hydroxysteroid dehydrogenase type IV); AI102103 (Pik4cb, Phosphatidylinositol 4-kinase); X56325 (Hba1, alpha 1 hemoglobin); X73371 (FCGR2, Low affinity immunoglobulin gamma Fc receptor II); X78848 (Gsta1, Glutathione-S-transferase (alpha type)); U92564 (Roaz, Olf-1/EBF associated Zn finger protein); AI171462 (Cd24, CD24 antigen); X83231 (PAIHC3, Pre-alpha-inhibitor, heavy chain 3); AF097593 (Ca4, cadherin 2- type 1 (neuronal)); X68283 (Rpl29, Ribosomal protein L29); S55427 (Pmp, peripheral myelin protein); AA818025 (Cd59, CD59 antigen); E01534 (Rps15, Ribosomal protein S15); U37138 (Sts, Steroid sulfatase); X55572 (Apod, Apolipoprotein D); AI028975 (AP-1, adaptor protein complex (beta 1)); L16995 (ADD1, adipocyte determination/ differentiation-dependent factor 1); U07971 (Transamidinase, Glycine amidinotransferase, mitochondrial); L07736 (Cpt1a, Carnitine palmitoyltransferase 1 alpha (liver)); AI237535 (LitaF, LPS-induced TNF-alpha factor); AI175486 (Rps7, Ribosomal protein S7); U32498 (RSEC8, rat homolog of yeast sec8); X53504 (RPL12, Ribosomal protein L12); AF023621 (Sort1, sortilin); AF083269 (P41-Arc, actin-related protein complex 1b); AA891810 (GST, Glutathione S-transferase); M77694 (Fah, fumarylacetoacetate hydrolase); M22357 (MAG, myelin-associated glycoprotein); AI230712 (Pace4, Subtilisin - like endoprotease); AF008439

(NRAMP2, Natural resistance-associated macrophage protein 2); U77829 (Gas-5, growth arrest homolog); U92081 (Gp38, Glycoprotein 38); AA891445 (Skd3, suppressor of K^+ transport defect 3); AI177161 (Nfe2l2, NF-E2-related factor 2); AF031430 (Stx7, Syntaxin 7); L35921 (Ggamma, GTP-binding protein (gamma subunit)); X62322 (Grn, Granulin); AF028784 (GFAP, glial fibrillary acidic protein); AI234146 (Csrp1, Cysteine rich protein 1) and mammalian homologues thereof.

33. The set of biomarkers of claim 23, wherein at least one member of the set of biomarkers is an expressed sequence tag (EST).

34. The set of biomarkers of claim 23, for use in the measurement of age-dependent cognitive decline.

35. The set of biomarkers of claim 34, wherein the age-dependent cognitive decline is an age-related neurodegenerative condition.

36. The set of biomarkers of claim 35, wherein the age-related neurodegenerative condition is Alzheimer's disease or Parkinson's disease.

37. The set of biomarkers of claim 23, for use in the measurement of degree of the safety or effectiveness of compounds or procedures directed against age-related cognitive decline.

38. A set of biomarkers for brain aging, comprising mammalian polynucleotides and polypeptides encoded by said polypeptides:
    (a) wherein the set of biomarkers comprises at least two members;
    (b) wherein the brain expression patterns of the members of the set are significantly altered with aging as measured by a conventional statistical method at a significance level of $p < 0.01$.

39. The set of biomarkers of claim 38, wherein the mammal is selected from the group consisting of rat, mouse and human.

40. The set of biomarkers of claim 38, wherein the conventional statistical method is ANOVA or student's t-test.

41. The set of biomarkers of claim 38, wherein at least one member of the set of biomarkers is a polynucleotide, or a polypeptide encoded by said polynucleotide, selected from the group consisting of AA891651 (rc_AA891651 EST195454 cDNA); AI070108 (rc_AI070108 UI-R-Y0-lu-a-09-0-UI.s1 cDNA); AI176689 (mitogen-activated protein kinase kinase 6); AI012051 (rc_AI012051 EST206502 cDNA); AI233365 (rc_AI233365 EST230053 cDNA); AA892532 (rc_AA892532 EST196335 cDNA); AA893185 (rc_AA893185 EST196988 cDNA); AA964320 (rc_AA964320 UI-R-C0-gu-e-09-0-UI.s1 cDNA); AA963449 (rc_AA963449 UI-R-E1-gj-e-08-0-UI.s1 cDNA); AA859632 (rc_AA859632 UI-R-E0-bs-h-08-0-UI.s1 cDNA); AI169265 (Atp6s1); AA850781 (rc_AA850781 EST193549 cDNA); AJ222813 (interleukin 18); D38380 (Transferrin); J03481 (dihydropteridine reductase); M24542 (Rieske iron-sulfur protein); L03294 (Lipoprotein lipase); L19998 (sulfotransferase family 1A, phenol-preferring, member 1); U53922 (DnaJ-like protein (RDJ1)); X54793 (liver heat shock protein (hsp60)); X62952 (vimentin); M55534 (Crystallin, alpha polypeptide 2); J03752 (microsomal glutathione S-transferase 1); X64401 (Cytochrome P450, subfamily IIIA, polypeptide 3); X78848 (Gsta1); AF016387 (retinoid X receptor gamma); AF031430 (syntaxin 7); AF051561 (solute carrier family 12, member 2); AF076183 (cytosolic sorting protein PACS-1a (PACS-1)); AF095576 (adaptor protein with pleckstrin homology and src homology 2 domains); AF095741 (MG87); AF097593 (cadherin 2, type 1, N-cadherin (neuronal)); AF104362 (osteoadherin); D10699 (ubiquitin carboxy-terminal hydrolase L1); D28111 (myelin-associated oligodendrocytic basic protein); D37951 (MIBP1 (c-myc intron binding protein 1)); D84477 (RhoA); L13202 (RATHFH2 HNF-3/fork-head homolog-2 (HFH-2)); L26292 (Kruppel-like factor 4 (gut)); L46873 (solute carrier family 15 (oligopeptide transporter), member 1); M13100 (RATLIN3A long interspersed repetitive DNA sequence LINE3 (L1Rn)); M27207 (procollagen, type I, alpha 1); M92433 (Zinc-finger transcription factor NGFI-C (early response gene)); M94918 (Hemoglobin, beta); M94919 (Hemoglobin, beta); S55427 (Peripheral

myelin protein); S68245 (carbonic anhydrase 4); S82649 (Narp=neuronal activity-regulated pentraxin); U10894 (allograft inflammatory factor 1); U26356 (RNSHUNA1 S100A1 gene); U75397 (RNKROX2 Krox-24); U75405 (procollagen, type I, alpha 1); U77829 (RNU77829 gas-5 growth arrest homolog non-translated sequence); U92081 (glycoprotein 38); X06554 (RNMAGSR myelin-associated glycoprotein (S-MAG) C-term); X13167 (Nuclear Factor IA); X14181 (RRRPL18A ribosomal protein L18a); X56325 (Hemoglobin, alpha 1); X60351 (Crystallin, alpha polypeptide 2); E13541 (chondroitin sulfate proteoglycan 5); M22357 (1B236/myelin-associated glycoprotein (MAG)); M24026 (RT1 class Ib gene); M24324 (MHC class I RT1 (RTS) (u haplotype)); J04488 (Prostaglandin D synthase); M15191 (Tachykinin (substance P, neurokinin A, neuropeptide K, neuropeptide gamma)); M74223 (VGF); U17254 (immediate early gene transcription factor NGFI-B); U08259 (Glutamate receptor, ionotropic, N-methyl D-aspartate 2C); U19866 (activity regulated cytoskeletal-associated protein); L40364 (RT1 class Ib gene); U17919 (allograft inflammatory factor 1); U78102 (early growth response 2); U67082 (KRAB-zinc finger protein KZF-1); U77777 (interleukin 18); D78018 (Nuclear Factor IA); U92564 (Olf-1/EBF associated Zn finger protein Roaz); AF008439 (Solute carrier family 11 member 2 (natural resistance-associated macrophage protein 2)); AB003726 (RuvB-like protein 1); M83561 (Glutamate receptor, ionotropic, kainate 1); AI639151 (mixed-tissue library cDNA clone rx02802 3); AI639247 (mixed-tissue library cDNA clone rx03939 3); AI639381 (mixed-tissue library cDNA clone rx01495 3); AI639532 (mixed-tissue library cDNA clone rx01030 3); AA799645 (FXYD domain-containing ion transport regulator 1); AA900516 (Pdi2); AI014169 (Vdup1); AI030089 (Nopp140); AI102299 (Bid3); AA818025 (CD59 antigen); AI170268 (Prostaglandin F receptor); AI171462 (CD24 antigen); AI171966 (ESTs, Highly similar to SPS2 MOUSE SELENIDE,WATER DIKINASE 2 [M.musculus]); AI176456 (ESTs, Weakly similar to ABP2_HUMAN ENDOTHELIAL ACTIN-BINDING PROTEIN [H.sapiens]); AI177161 (NF-E2-related factor 2); AI179576 (Hemoglobin, beta); AI230712 (Subtilisin - like endoprotease); AI230914 (farnesyltransferase beta subunit); AI231213 (kangai 1 (suppression of tumorigenicity 6), prostate); AI237731 (Lipoprotein lipase); M83745 (Protein convertase subtilisin / kexin, type I); M27905 (ribosomal protein L21);

M32016 (Lysosomal-associated membrane protein 2); M11071 (RT1 class Ib gene); M15562 (MHC class II RT1.u-D-alpha chain); M15880 (Neuropeptide Y); L08595 (nuclear receptor subfamily 4, group A, member 2); M18416 (Early growth response 1); L40362 (MHC class I RT1.C-type protein); Z50144 (kynurenine/alpha-aminoadipate aminotransferase); X71127 (complement component 1, q subcomponent, beta polypeptide); U44948 (smooth muscle cell LIM protein (SmLIM)); AA850219 (Annexin A3); X73371 (FCGR2); X57281 (Glycine receptor alpha 2 subunit (glycine receptor, neonatal)); X83231 (pre-alpha-inhibitor); X52477 (Complement component 3); X16554 (phosphoribosyl pyrophosphate synthetase 1); X78605 ((Sprague Dawley) rab4b ras-homologous GTPase); X82445 (nuclear distribution gene C homolog (Aspergillus)); X52619 (ribosomal protein L28); X68283 (ribosomal protein L29); X13044 (CD74 antigen (invariant polpypeptide of major histocompatibility class II antigen-associated)); X54096 (Lecithin-cholesterol acyltransferase); U31866 (Nclone10); U72620 (Lot1); U66470 (rCGR11); M31018 (RT1 class Ib gene); U90887 (arginase type II); M18467 (Glutamate oxaloacetate transaminase 2, mitochondrial (aspartate aminotransferase 2)); M64780 (Agrin); U87627 (putative monocarboxylate transporter (MCT3)); AF019974 (Chromogranin B, parathyroid secretory protein); L03201 (cathepsin S); AB008538 (HB2); D89340 (dipeptidylpeptidase III); M77694 (fumarylacetoacetate hydrolase); M32062 (Fc-gamma receptor); L21192 (brain abundant, membrane attached signal protein 2); M37584 (H2afz); AA858588 (ESTs, Weakly similar to ODP2 RAT DIHYDROLIPOAMIDE ACETYLTRANSFERASE COMPONENT OF PYRUVATE DEHYDROGENASE COMPLEX [R.norvegicus]); AA858617 (rc_AA858617 UI-R-E0-bq-b-06-0-UI.s1 cDNA); AA859562 (rc_AA859562 UI-R-E0-bv-b-03-0-UI.s1 cDNA); AA859626 (rc_AA859626 UI-R-E0-bs-h-02-0-UI.s1 cDNA); AA859690 (rc_AA859690 UI-R-E0-bx-e-11-0-UI.s1 cDNA); AA859777 (rc_AA859777 UI-R-E0-bu-e-10-0-UI.s1 cDNA); AA859975 (LOC64201); AA860030 (UI-R-E0-bz-e-07-0-UI.s2 cDNA); AA866291 (rc_AA866291 UI-R-A0-ac-e-12-0-UI.s3 cDNA); AA866409 (rc_AA866409 UI-R-E0-ch-a-03-0-UI.s1 cDNA); AA866411 (NDN); AA874794 (Bex3); A874887 (rc_AA874887 UI-R-E0-ci-g-10-0-UI.s1 cDNA); AA875004 (rc_AA875004 UI-R-E0-cb-b-07-0-UI.s1 cDNA); AA875037 (rc_AA875037 UI-R-E0-cb-a-03-0-UI.s1 cDNA); AA875047

(TCPZ); AA875059 (rc_AA875059 UI-R-E0-cb-f-04-0-UI.s1 cDNA); AA875129 (rc_AA875129 UI-R-E0-bu-e-01-0-UI.s2 cDNA); H31418 (rc_H31418 EST105434 cDNA); H31665 (rc_H31665 EST105952 cDNA); H32977 (rc_H32977 EST108553 cDNA); H33725 (associated molecule with the SH3 domain of STAM); AA891037 (rc_AA891037 EST194840 cDNA); AA891445 (Skd3); AA891690 (ESTs, Weakly similar to SERC_HUMAN PHOSPHOSERINE AMINOTRANSFERASE [H.sapiens]); AA891717 (USF1); AA891734 (rc_AA891734 EST195537 cDNA); AA891785 (rc_AA891785 EST195588 cDNA); AA891810 (ESTs, Highly similar to GTK1 RAT GLUTATHIONE S-TRANSFERASE, MITOCHONDRIAL [R.norvegicus]); AA891965 (rc_AA891965 EST195768 cDNA); AA892333 (Tuba1); AA892353 (ESTs, Moderately similar to JC5823 NADH dehydrogenase [H.sapiens]); AA892511 (rc_AA892511 EST196314 cDNA); AA892986 (rc_AA892986 EST196789 cDNA); AA893032 (ESTs, Moderately similar to CALX RAT CALNEXIN PRECURSOR [R.norvegicus]); AA893082 (rc_AA893082 EST196885 cDNA); AA893493 (RPL26); AA893607 (rc_AA893607 EST197410 cDNA); AA893708 (KIAA0560); AA893743 (rc_AA893743 EST197546 cDNA); AA894104 (rc_AA894104 EST197907 cDNA); AA799449 (EST, Weakly similar to UBP4 MOUSE UBIQUITIN CARBOXYL-TERMINAL HYDROLASE 4 [M.musculus]); AA799779 (acyl-CoA:dihydroxyacetonephosphate acyltransferase); AA799803 (ESTs, Weakly similar to K1CU RAT KERATIN, TYPE I CYTOSKELETAL 21 [R.norvegicus]); AA799854 (rc_AA799854 EST189351 cDNA); AA799996 (rc_AA799996 EST189493 cDNA); AA800693 (rc_AA800693 EST190190 cDNA); AA800708 (ESTs, Weakly similar to S28312 hypothetical protein F02A9.4 - Caenorhabditis elegans [C.elegans]); AA800794 (HT2A); AA800948 (Tuba4); and mammalian homologues thereof.

42. The set of biomarkers of claim 38, wherein at least one member of the set of biomarkers is an expressed sequence tag (EST).

43. The set of biomarkers of claim 38, for use in the measurement of age-dependent cognitive decline.

44. The set of biomarkers of claim 43, wherein the age-dependent cognitive decline is an age-dependent neurodegenerative condition.

45. The set of biomarkers of claim 44, wherein the age-dependent neurodegenerative condition is Alzheimer's disease or Parkinson's disease.

46. The set of biomarkers of claim 38, for use in the measurement of the degree of the safety or effectiveness of compounds or procedures directed against age-related cognitive decline.

47. The use of an expressed sequence tag (EST) in an assay for aging in a mammal, wherein the EST is selected from the group consisting of AA963449 (UI-R-E1-gj-e-08-0-UI.s1 cDNA); AA892532 (EST196335 cDNA); AA859626 (UI-R-E0-bs-h-02-0-UI.s1 cDNA); AA893743 (EST197546 cDNA); AI233365 (EST230053 cDNA); H31665 (EST105952 cDNA); AA892353 (ESTs, Moderately similar to JC5823 NADH dehydrogenase); AI639247 (mixed-tissue library cDNA clone rx03939 3); AA858617 (UI-R-E0-bq-b-06-0-UI.s1 cDNA); AI639429 (mixed-tissue library cDNA clone rx00973 3); AA858620 (UI-R-E0-bq-b-09-0-UI.s1 cDNA); AA866291 (UI-R-A0-ac-e-12-0-UI.s3 cDNA); AA894104 (EST197907 cDNA); AA799996 (EST189493 cDNA); AA892805 (EST196608 cDNA); AI639019 (mixed-tissue library cDNA clone rx01107 3); AA799538 (EST189035 cDNA); AI070108 (UI-R-Y0-lu-a-09-0-UI.s1 cDNA); AA866409 (UI-R-E0-ch-a-03-0-UI.s1 cDNA); AA859632 (UI-R-E0-bs-h-08-0-UI.s1 cDNA); AA891651 (EST195454 cDNA); AA893032 (ESTs, Moderately similar to CALX calnexin precursor); AA891965 (EST195768 cDNA); AA800708 (ESTs, Weakly similar to S28312 hypothetical protein F02A9.4); AA964320 (UI-R-C0-gu-e-09-0-UI.s1 cDNA); AA893173 (EST196976 cDNA); H32977 (EST108553 cDNA); AA874887 (UI-R-E0-ci-g-10-0-UI.s1 cDNA); AA850781 (EST193549 cDNA); AI176456 (ESTs, Weakly similar to endothelial actin-binding protein); H31418 (EST105434 cDNA); AA858588 (ESTs, Weakly similar to ODP2 dihydrolipoamide acetyl transferase); AA891785 (EST195588 cDNA); AA799803 (ESTs, Weakly similar to K1CU cytoskeletal keratin (type 1)); AA799449 (EST, Weakly similar to UBP4 ubiquitin carboxyl-terminal

hydrolase 4); AA859777 (UI-R-E0-bu-e-10-0-UI.s1 cDNA); AI639532 (mixed-tissue library cDNA clone rx01030 3); AA875059 (UI-R-E0-cb-f-04-0-UI.s1 cDNA); AI012051 (EST206502 cDNA); AA800549 (EST190046 cDNA); AA799854 (EST189351 cDNA); AA892520 (EST196323 cDNA) and mammalian homologues thereof.

48. The use of claim 47, wherein the assay for aging is a measurement of age-dependent cognitive decline.

49. The use of claim 48, wherein the age-dependent cognitive decline is an age-dependent neurodegenerative condition.

50. The use of claim 49, wherein the age-dependent neurodegenerative condition is Alzheimer's disease or Parkinson's disease.

51. The use of claim 47, wherein the assay for aging is a measurement of the degree of the safety or effectiveness of compounds or procedures directed against age-related cognitive decline.

52. A set of biomarkers for brain aging, comprising mammalian polynucleotides and polypeptides encoded by said polynucleotides:
    (a) wherein the set of biomarkers comprises at least two members; and
    (b) wherein the brain expression patterns of the members of the set are significantly altered with aging as determined by a conventional statistical method, with $p < 0.05$.

53. The set of biomarkers of claim 52, wherein the set contains at least one member selected from the group consisting of AA685974 (EST108806 cDNA); AA799396 (EST188893 cDNA); AA799479 (ESTs, Highly similar to NADH-ubiquinone oxidoreduct.); AA799481 (EST188978 cDNA); AA799529 (EST189026 cDNA); AA799599 (EST189096 cDNA); AA799636 (EST189133 cDNA); AA799680 (EST189177 cDNA); AA799724 (ESTs, Highly similar to DNA-directed RNA

polymeraseI); AA799773 (EST189270 cDNA); AA799779 (acyl-CoA:dihydroxyacetonephosphate acyltransferase); AA799858 (EST189355 cDNA); AA800026 (EST189523 cDNA); AA800318 (EST189815 cDNA); AA800622 (EST190119 cDNA); AA800693 (EST190190 cDNA); AA800948 (Tuba4); AA801286 (Inositol (myo)-1(or 4)-monophosphatase 1); AA817887 (profilin); AA818025 (CD59 antigen); AA818240 (Nuclear pore complex protein); AA818487 (cyclophilin B); AA819500 (ESTs, Highly similar to AC12_HUMAN 37 kD subunit); AA819708 (Cox7a3); AA848831 (lysophosphatidic acid G-protein-coupled receptor, 2); AA852046 (EST194815 cDNA); AA859545 (ESTs, Weakly similar to hypothetical protein C09H6.3); AA859562 (UI-R-E0-bv-b-03-0-UI.s1 cDNA); AA859643 (UI-R-E0-bs-a-08-0-UI.s1 cDNA); AA859645 (attractin); AA859690 (UI-R-E0-bx-e-11-0-UI.s1 cDNA); AA859848 (UI-R-E0-cc-h-10-0-UI.s1 cDNA); AA859954 (Vacuole Membrane Protein 1); AA859980 (T-complex 1); AA860030 (UI-R-E0-bz-e-07-0-UI.s2 cDNA); AA866257 (ESTs); AA866299 (UI-R-A0-ac-f-12-0-UI.s3 cDNA); AA866299 (UI-R-A0-ac-f-12-0-UI.s3 cDNA); AA866432 (UI-R-E0-ch-e-06-0-UI.s1 cDNA); AA866477 (UI-R-E0-br-h-03-0-UI.s1 cDNA); AA874830 (UI-R-E0-cg-f-04-0-UI.s1 cDNA); AA874874 (ESTs, Highly similar to alcohol dehydrogenase class III); AA874969 (ESTs, Highly similar to c-Jun leucine zipper interactive); AA874995 (UI-R-E0-cf-d-08-0-UI.s1 cDNA); AA875004 (UI-R-E0-cb-b-07-0-UI.s1 cDNA); AA875019 (UI-R-E0-cb-f-08-0-UI.s1 cDNA); AA875032 (UI-R-E0-cb-h-09-0-UI.s1 cDNA); AA875037 (UI-R-E0-cb-a-03-0-UI.s1 cDNA); AA875129 (UI-R-E0-bu-e-01-0-UI.s2 cDNA); AA875257 (UI-R-E0-cq-d-12-0-UI.s1 cDNA); AA891037 (EST194840 cDNA); AA891041 (jun B proto-oncogene); AA891221 (EST195024 cDNA); AA891476 (EST195279 cDNA); AA891537 (EST195340 cDNA); AA891690 (ESTs, Weakly similar to p-serine aminotransferase); AA891727 (EST195530 cDNA); AA891734 (EST195537 cDNA); AA891774 (EST195577 cDNA); AA891810 (EST195613 cDNA); AA891880 (Loc65042); AA891916 (membrane interacting protein of RGS16); AA891944 (EST195747 cDNA); AA891950 (EST195753 cDNA); AA892146 (EST195949 cDNA); AA892298 (EST196101 cDNA); AA892414 (EST196217 cDNA); AA892511 (EST196314 cDNA); AA892520 (EST196323 cDNA); AA892538 (EST196341 cDNA); AA892637 (EST196440 cDNA); AA892775 (Lysozyme);

AA892796 (EST196599 cDNA); AA892813 (EST196616 cDNA); AA892986 (EST196789 cDNA); AA893082 (EST196885 cDNA); AA893185 (EST196988 cDNA); AA893199 (EST197002 cDNA); AA893224 (EST197027 cDNA); AA893320 (EST197123 cDNA); AA893584 (EST197387 cDNA); AA893690 (EST197493 cDNA); AA893708 (KIAA0560); AA893717 (EST197520 cDNA); AA893743 (EST197546 cDNA); AA893788 (ESTs, Highly similar to chromobox protein homolog 5); AA893946 (EST197749 cDNA); AA894305 (EST198108 cDNA); AA924925 (ER transmembrane protein Dri 42); AA942685 (cytosolic cysteine dioxygenase 1); AA955306 (ras-related protein rab10); AA955388 (Na^+K^+ transporting ATPase 2, beta polypeptide 2); AA957132 (N-acetylglucosaminyltransferase I); AB000778 (Phoshpolipase D gene 1); AB006451 (Tim23); AB008538 (HB2); AB016532 (period homolog 2 (Drosophila)); AF000899 (p58/p45, nucleolin); AF007554 (Mucin1); AF007758 (synuclein, alpha); AF007890 (resection-induced TPI (rs11)); AF008554 (implantation-associated protein (IAG2)); AF013144 (MAP-kinase phosphatase (cpg21)); AF016269 (kallikrein 6 (neurosin, zyme)); AF016296 (neuropilin); AF019974 (Chromogranin B, parathyroid secretory protein); AF020046 (integrin alpha E1, epithelial-associated); AF021935 (Ser-Thr protein kinase); AF023087 (Early growth response 1); AF030050 (replication factor C); AF030088 (RuvB-like protein 1); AF040954 (putative protein phosphatase1 nuclear targeting subunit); AF051561 (solute carrier family 12, member 2); AF055477 (L-type voltage-dependent Ca^{2+} channel (?1D subunit)); AF074608 (MHC class I antigen (RT1.EC2) gene); AF076183 (cytosolic sorting protein PACS-1a (PACS-1)); AF095927 (protein phosphatase 2C); AI010110 (SH3-domain GRB2-like 1); AI012589 (glutathione S-transferase, pi 2); AI013627 (defender against cell death 1); AI013861 (3-hydroxyisobutyrate dehydrogenase); AI045249 (heat shock 70kD protein 8); AI102031 (myc box dependent interacting protein 1); AI102299 (Bid3); AI102839 (cerebellar Ca-binding protein, spot 35 protein); AI102868 (EST212157 cDNA); AI104388 (heat shock 27kD protein 1); AI136891 (zinc finger protein 36, C3H type-like 1); AI168942 (branched chain keto acid dehydrogenase E1); AI169265 (Atp6s1); AI171966 (ESTs, Highly similar to selenide, water dikinase 2); AI175973 (ESTs, Highly similar to NADH dehydrogenase); AI176491 (EST220076 cDNA); AI176595 (Cathepsin L); AI176621 (iron-responsive element-binding protein);

cDNA clone rx00973 3); AA858620 (UI-R-E0-bq-b-09-0-UI.s1 cDNA); AA866291 (UI-R-A0-ac-e-12-0-UI.s3 cDNA); AA894104 (EST197907 cDNA); AA799996 (EST189493 cDNA); AA892805 (EST196608 cDNA); AI639019 (mixed-tissue library cDNA clone rx01107 3); AA799538 (EST189035 cDNA); AI070108 (UI-R-Y0-lu-a-09-0-UI.s1 cDNA); AA866409 (UI-R-E0-ch-a-03-0-UI.s1 cDNA); AA859632 (UI-R-E0-bs-h-08-0-UI.s1 cDNA); AA891651 (EST195454 cDNA); AA893032 (ESTs, Moderately similar to CALX calnexin precursor); AA891965 (EST195768 cDNA); AA800708 (ESTs, Weakly similar to S28312 hypothetical protein F02A9.4); AA964320 (UI-R-C0-gu-e-09-0-UI.s1 cDNA); AA893173 (EST196976 cDNA); H32977 (EST108553 cDNA); AA874887 (UI-R-E0-ci-g-10-0-UI.s1 cDNA); AA850781 (EST193549 cDNA); AI176456 (ESTs, Weakly similar to endothelial actin-binding protein); H31418 (EST105434 cDNA); AA858588 (ESTs, Weakly similar to ODP2 dihydrolipoamide acetyl transferase); AA891785 (EST195588 cDNA); AA799803 (ESTs, Weakly similar to K1CU cytoskeletal keratin (type 1)); AA799449 (EST, Weakly similar to UBP4 ubiquitin carboxyl-terminal hydrolase 4); AA859777 (UI-R-E0-bu-e-10-0-UI.s1 cDNA); AI639532 (mixed-tissue library cDNA clone rx01030 3); AA875059 (UI-R-E0-cb-f-04-0-UI.s1 cDNA); AI012051 (EST206502 cDNA); AA800549 (EST190046 cDNA); AA799854 (EST189351 cDNA); AA892520 (EST196323 cDNA) and mammalian homologues thereof.

48. The use of claim 47, wherein the assay for aging is a measurement of age-dependent cognitive decline.

49. The use of claim 48, wherein the age-dependent cognitive decline is an age-dependent neurodegenerative condition.

50. The use of claim 49, wherein the age-dependent neurodegenerative condition is Alzheimer's disease or Parkinson's disease.

51. The use of claim 47, wherein the assay for aging is a measurement of the degree of the safety or effectiveness of compounds or procedures directed against age-related cognitive decline.

52. A set of biomarkers for brain aging, comprising mammalian polynucleotides and polypeptides encoded by said polynucleotides:

(a) wherein the set of biomarkers comprises at least two members; and

(b) wherein the brain expression patterns of the members of the set are significantly altered with aging as determined by a conventional statistical method, with $p < 0.05$.

53. The set of biomarkers of claim 52, wherein the set contains at least one member selected from the group consisting of AA685974 (EST108806 cDNA); AA799396 (EST188893 cDNA); AA799479 (ESTs, Highly similar to NADH-ubiquinone oxidoreduct.); AA799481 (EST188978 cDNA); AA799529 (EST189026 cDNA); AA799599 (EST189096 cDNA); AA799636 (EST189133 cDNA); AA799680 (EST189177 cDNA); AA799724 (ESTs, Highly similar to DNA-directed RNA polymerasel); AA799773 (EST189270 cDNA); AA799779 (acyl-CoA:dihydroxyacetonephosphate acyltransferase); AA799858 (EST189355 cDNA); AA800026 (EST189523 cDNA); AA800318 (EST189815 cDNA); AA800622 (EST190119 cDNA); AA800693 (EST190190 cDNA); AA800948 (Tuba4); AA801286 (Inositol (myo)-1(or 4)-monophosphatase 1); AA817887 (profilin); AA818025 (CD59 antigen); AA818240 (Nuclear pore complex protein); AA818487 (cyclophilin B); AA819500 (ESTs, Highly similar to AC12_HUMAN 37 kD subunit); AA819708 (Cox7a3); AA848831 (lysophosphatidic acid G-protein-coupled receptor, 2); AA852046 (EST194815 cDNA); AA859545 (ESTs, Weakly similar to hypothetical protein C09H6.3); AA859562 (UI-R-E0-bv-b-03-0-UI.s1 cDNA); AA859643 (UI-R-E0-bs-a-08-0-UI.s1 cDNA); AA859645 (attractin); AA859690 (UI-R-E0-bx-e-11-0-UI.s1 cDNA); AA859848 (UI-R-E0-cc-h-10-0-UI.s1 cDNA); AA859954 (Vacuole Membrane Protein 1); AA859980 (T-complex 1); AA860030 (UI-R-E0-bz-e-07-0-UI.s2 cDNA); AA866257 (ESTs); AA866299 (UI-R-A0-ac-f-12-

0-UI.s3 cDNA); AA866299 (UI-R-A0-ac-f-12-0-UI.s3 cDNA); AA866432 (UI-R-E0-ch-e-06-0-UI.s1 cDNA); AA866477 (UI-R-E0-br-h-03-0-UI.s1 cDNA); AA874830 (UI-R-E0-cg-f-04-0-UI.s1 cDNA); AA874874 (ESTs, Highly similar to alcohol dehydrogenase class III); AA874969 (ESTs, Highly similar to c-Jun leucine zipper interactive); AA874995 (UI-R-E0-cf-d-08-0-UI.s1 cDNA); AA875004 (UI-R-E0-cb-b-07-0-UI.s1 cDNA); AA875019 (UI-R-E0-cb-f-08-0-UI.s1 cDNA); AA875032 (UI-R-E0-cb-h-09-0-UI.s1 cDNA); AA875037 (UI-R-E0-cb-a-03-0-UI.s1 cDNA); AA875129 (UI-R-E0-bu-e-01-0-UI.s2 cDNA); AA875257 (UI-R-E0-cq-d-12-0-UI.s1 cDNA); AA891037 (EST194840 cDNA); AA891041 (jun B proto-oncogene); AA891221 (EST195024 cDNA); AA891476 (EST195279 cDNA); AA891537 (EST195340 cDNA); AA891690 (ESTs, Weakly similar to p-serine aminotransferase); AA891727 (EST195530 cDNA); AA891734 (EST195537 cDNA); AA891774 (EST195577 cDNA); AA891810 (EST195613 cDNA); AA891880 (Loc65042); AA891916 (membrane interacting protein of RGS16); AA891944 (EST195747 cDNA); AA891950 (EST195753 cDNA); AA892146 (EST195949 cDNA); AA892298 (EST196101 cDNA); AA892414 (EST196217 cDNA); AA892511 (EST196314 cDNA); AA892520 (EST196323 cDNA); AA892538 (EST196341 cDNA); AA892637 (EST196440 cDNA); AA892775 (Lysozyme); AA892796 (EST196599 cDNA); AA892813 (EST196616 cDNA); AA892986 (EST196789 cDNA); AA893082 (EST196885 cDNA); AA893185 (EST196988 cDNA); AA893199 (EST197002 cDNA); AA893224 (EST197027 cDNA); AA893320 (EST197123 cDNA); AA893584 (EST197387 cDNA); AA893690 (EST197493 cDNA); AA893708 (KIAA0560); AA893717 (EST197520 cDNA); AA893743 (EST197546 cDNA); AA893788 (ESTs, Highly similar to chromobox protein homolog 5); AA893946 (EST197749 cDNA); AA894305 (EST198108 cDNA); AA924925 (ER transmembrane protein Dri 42); AA942685 (cytosolic cysteine dioxygenase 1); AA955306 (ras-related protein rab10); AA955388 (Na^+K^+ transporting ATPase 2, beta polypeptide 2); AA957132 (N-acetylglucosaminyltransferase I); AB000778 (Phoshpolipase D gene 1); AB006451 (Tim23); AB008538 (HB2); AB016532 (period homolog 2 (Drosophila)); AF000899 (p58/p45, nucleolin); AF007554 (Mucin1); AF007758 (synuclein, alpha); AF007890

(resection-induced TPI (rs11)); AF008554 (implantation-associated protein (IAG2)); AF013144 (MAP-kinase phosphatase (cpg21)); AF016269 (kallikrein 6 (neurosin, zyme)); AF016296 (neuropilin); AF019974 (Chromogranin B, parathyroid secretory protein); AF020046 (integrin alpha E1, epithelial-associated); AF021935 (Ser-Thr protein kinase); AF023087 (Early growth response 1); AF030050 (replication factor C); AF030088 (RuvB-like protein 1); AF040954 (putative protein phosphatase1 nuclear targeting subunit); AF051561 (solute carrier family 12, member 2); AF055477 (L-type voltage-dependent Ca^{2+} channel (?1D subunit)); AF074608 (MHC class I antigen (RT1.EC2) gene); AF076183 (cytosolic sorting protein PACS-1a (PACS-1)); AF095927 (protein phosphatase 2C); AI010110 (SH3-domain GRB2-like 1); AI012589 (glutathione S-transferase, pi 2); AI013627 (defender against cell death 1); AI013861 (3-hydroxyisobutyrate dehydrogenase); AI045249 (heat shock 70kD protein 8); AI102031 (myc box dependent interacting protein 1); AI102299 (Bid3); AI102839 (cerebellar Ca-binding protein, spot 35 protein); AI102868 (EST212157 cDNA); AI104388 (heat shock 27kD protein 1); AI136891 (zinc finger protein 36, C3H type-like 1); AI168942 (branched chain keto acid dehydrogenase E1); AI169265 (Atp6s1); AI171966 (ESTs, Highly similar to selenide, water dikinase 2); AI175973 (ESTs, Highly similar to NADH dehydrogenase); AI176491 (EST220076 cDNA); AI176595 (Cathepsin L); AI176621 (iron-responsive element-binding protein); AI177404 (EST221024 cDNA); AI178204 (EST221869 cDNA); AI178921 (Insulin degrading enzyme); AI228548 (ESTs, Highly similar to DKFZp586G0322.1); AI230247 (selenoprotein P, plasma, 1); AI230778 (ESTs, Highly similar to protein-tyrosine sulfotrans. 2); AI230914 (farnesyltransferase beta subunit); AI231807 (ferritin light chain 1); AI231807 (ferritin light chain 1); AI232268 (LDL receptor-related protein associated protein 1); AI235344 (geranylgeranyltransferase type I (GGTase-I)); AI237007 (ESTs, Highly similar to flavoprot.-ubiquin. Oxidoreduct.); AI638971 (mixed-tissue library cDNA clone rx04989 3); AI638997 (mixed-tissue library cDNA clone rx05048 3); AI639151 (mixed-tissue library cDNA clone rx02802 3); AI639209 (mixed-tissue library cDNA clone rx00680 3); AI639257 (mixed-tissue library cDNA clone rx01119 3); AI639477 (mixed-tissue library cDNA clone rx02351 3); D00569 (2,4-dienoyl CoA reductase 1, mitochondrial); D10262

(choline kinase); D10699 (ubiquitin carboxy-terminal hydrolase L1); D10854 (aldehyde reductase); D10874 (lysosomal vacuolar proton pump (16 kDa)); D16478 (mitochondrial long-chain enoyl-CoA hydratase); D17309 (delta 4-3-ketosteroid-5-beta-reductase); D28110 (myelin-associated oligodendrocytic basic protein); D28110 (myelin-associated oligodendrocytic basic protein); D28557 (cold shock domain protein A); D29766 (v-crk-associated tyrosine kinase substrate); D37951 (MIBP1 (c-myc intron binding protein 1)); D45247 (proteasome subunit RCX); D45249 (protease (prosome, macropain) 28 subunit, alpha); D78308 (calreticulin); D83948 (adult liver S1-1 protein); D88586 (eosinophil cationic protein); D89340 (dipeptidylpeptidase III); D89730 (Fibulin 3, fibulin-like extracellular matrix protein 1); D90211 (Lysosomal-associated membrane protein 2); E03229 (cytosolic cysteine dioxygenase 1); H33086 (EST108750 cDNA); H33725 (associated molecule with the SH3 domain of STAM); J02752 (acyl-coA oxidase); J02773 (heart fatty acid binding protein); J05022 (peptidylarginine deiminase); J05031 (Isovaleryl Coenzyme A dehydrogenase); J05132 (UDP-glucuronosyltransferase); K02248 (Somatostatin); L00191 (Fibronectin 1); L13202 (RATHFH2 HNF-3/fork-head homolog-2 (HFH-2)); L19998 (sulfotransferase family 1A, phenol-preferring, member 1); L24896 (glutathione peroxidase 4); L25605 (Dynamin 2); L26292 (Kruppel-like factor 4 (gut)); L29573 (neurotransmitter transporter,noradrenalin); L42855 (transcription elongation factor B (SIII) polypeptide 2); M10068 (NADPH-cytochrome P-450 oxidoreductase); M13100 (long interspersed repetitive DNA sequence LINE3); M13100 (long interspersed repetitive DNA sequence LINE3); M19936 (Prosaposin-sphingolipid hydrolase activator); M23601 (Monoamine oxidase B); M24104 (synaptobrevin 2); M24104 (Vesicle-associated membrane protein (synaptobrevin 2)); M24852 (Neuron specific protein PEP-19 (Purkinje cell protein 4)); M31174 (thyroid hormone receptor alpha); M36453 (Inhibin, alpha); M55015 (nucleolin); M57276 (Leukocyte antigen (Ox-44)); M58404 (thymosin, beta 10); M80550 (adenylyl cyclase); M83745 (Protein convertase subtilisin / kexin, type I); M89646 (ribosomal protein S24); M91234 (VL30 element); M93273 (somatostatin receptor subtype 2); M93669 (Secretogranin II); M99485 (Myelin oligodendrocyte glycoprotein); S53527 (S100 calcium-binding protein, beta (neural)); S61868 (Ryudocan/syndecan 4);

S72594 (tissue inhibitor of metalloproteinase 2); S77492 (Bone morphogenetic protein 3); S77858 (non-muscle myosin alkali light chain); U04738 (Somatostatin receptor subtype 4); U07619 (Coagulation factor III (thromboplastin, tissue factor)); U08259 (Glutamate receptor, N-methyl D-aspartate 2C); U10357 (pyruvate dehydrogenase kinase 2 subunit p45 (PDK2)); U14950 (tumor suppressor homolog (synapse associ. protein)); U17254 (immediate early gene transcription factor NGFI-B); U17254 (immediate early gene transcription factor NGFI-B); U18771 (Ras-related protein Rab-26); U27518 (UDP-glucuronosyltransferase); U28938 (receptor-type protein tyrosine phosphatase D30); U38379 (Gamma-glutamyl hydrolase); U38801 (DNA polymerase beta); U67080 (r-MyT13); U67136 (A kinase (PRKA) anchor protein 5); U67137 (guanylate kinase associated protein); U72620 (Lot1); U75405 (procollagen, type I, alpha 1); U75917 (clathrin-associated protein 17); U77777 (interleukin 18); U78517 (cAMP-regulated guanine nucleotide exchange factor II); U89905 (alpha-methylacyl-CoA racemase); V01244 (Prolactin); X02904 (glutathione S-transferase P subunit); X05472 (2.4 kb repeat DNA right terminal region); X06769 (FBJ v-fos oncogene homolog); X06916 (S100 calcium-binding protein A4); X13905 (ras-related rab1B protein); X14323 (Fc receptor, IgG, alpha chain transporter); X16933 (RNA binding protein p45AUF1); X53427 (glycogen synthase kinase 3 alpha (EC 2.7.1.37)); X53504 (ribosomal protein L12); X54467 (cathepsin D); X55153 (ribosomal protein P2); X57281 (Glycine receptor alpha 2 subunit); X58294 (carbonic anhydrase 2); X59737 (ubiquitous mitochondrial creatine kinase); X60212 (ASI homolog of bacterial ribosomal subunit protein L22); X62950 (pBUS30 with repetitive elements); X67805 (Synaptonemal complex protein 1); X72757 (cox VIa gene (liver)); X74226 (LL5 protein); X76489 (CD9 cell surface glycoprotein); X76985 (latexin); X82445 (nuclear distribution gene C homolog (Aspergillus)); X84039 (lumican); X89696 (TPCR06 protein); X97443 (integral membrane protein Tmp21-I (p23)); X98399 (solute carrier family 14, member 1); Y17295 (thiol-specific antioxidant protein (1-Cys peroxiredoxin)); Z48225 (protein synthesis initiation factor eIF-2B delta subunit); Z49858 (plasmolipin) and mammalian homologues.

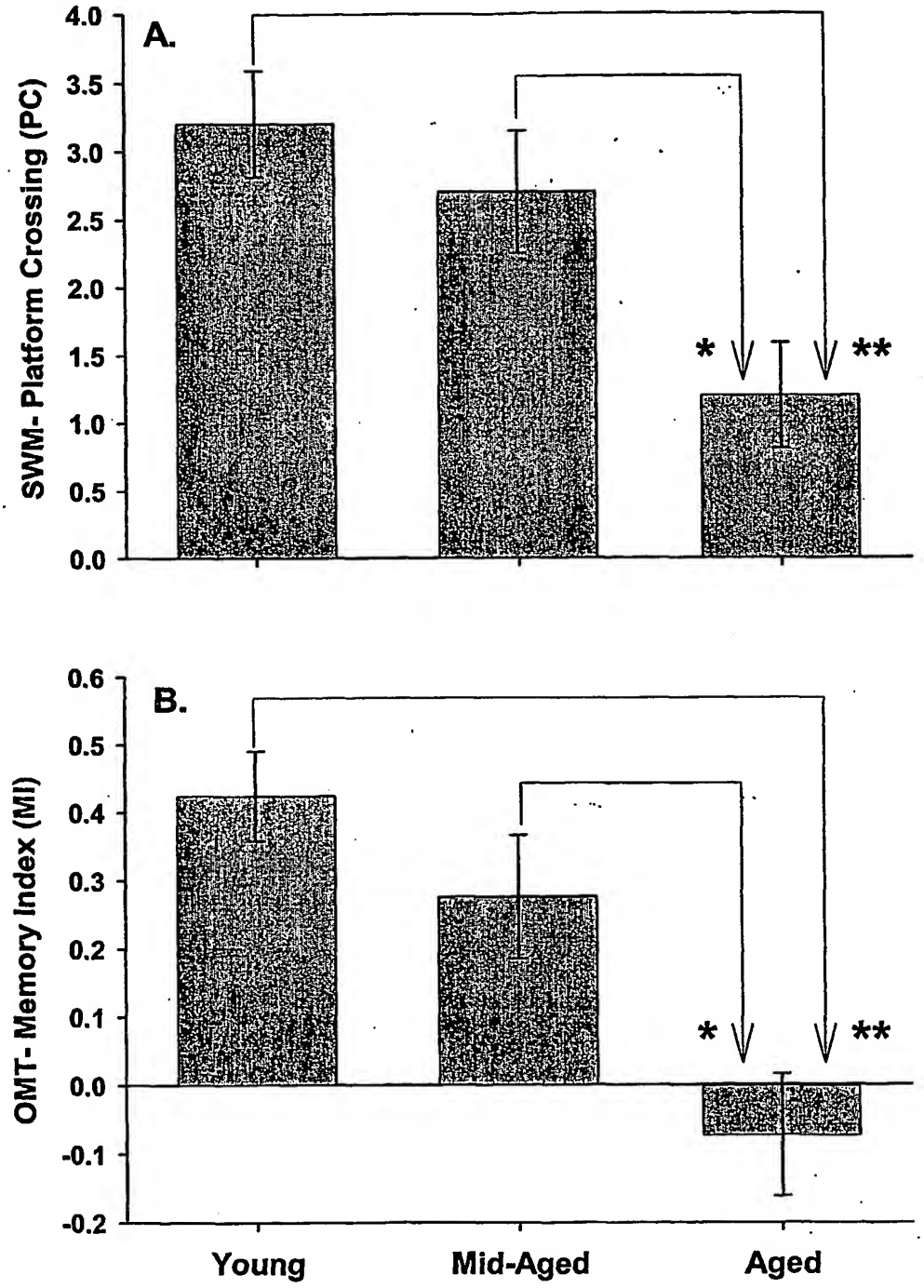
A.
SWM- Platform Crossing (PC)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
**
B.
OMT- Memory Index (MI)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
*
**
Young
Mid-Aged
Aged

FIG. 1

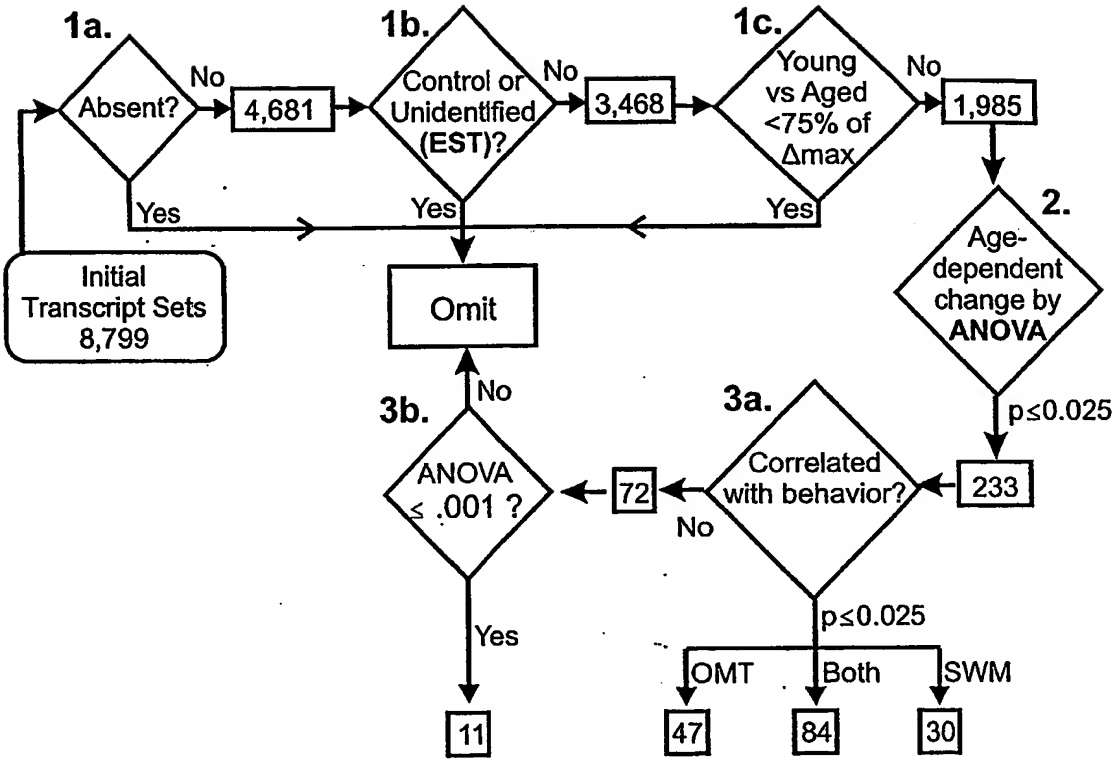
1a.
Absent?
No
4,681
1b.
Control or Unidentified (EST)?
No
3,468
1c.
Young vs Aged <75% of Δmax
No
1,985
2.
Age-dependent change by ANOVA
p≤0.025
233
Yes
Yes
Yes
Initial Transcript Sets 8,799
Omit
3b.
ANOVA ≤ .001 ?
No
72
3a.
Correlated with behavior?
No
p≤0.025
OMT
Both
SWM
Yes
11
47
84
30

FIG. 2

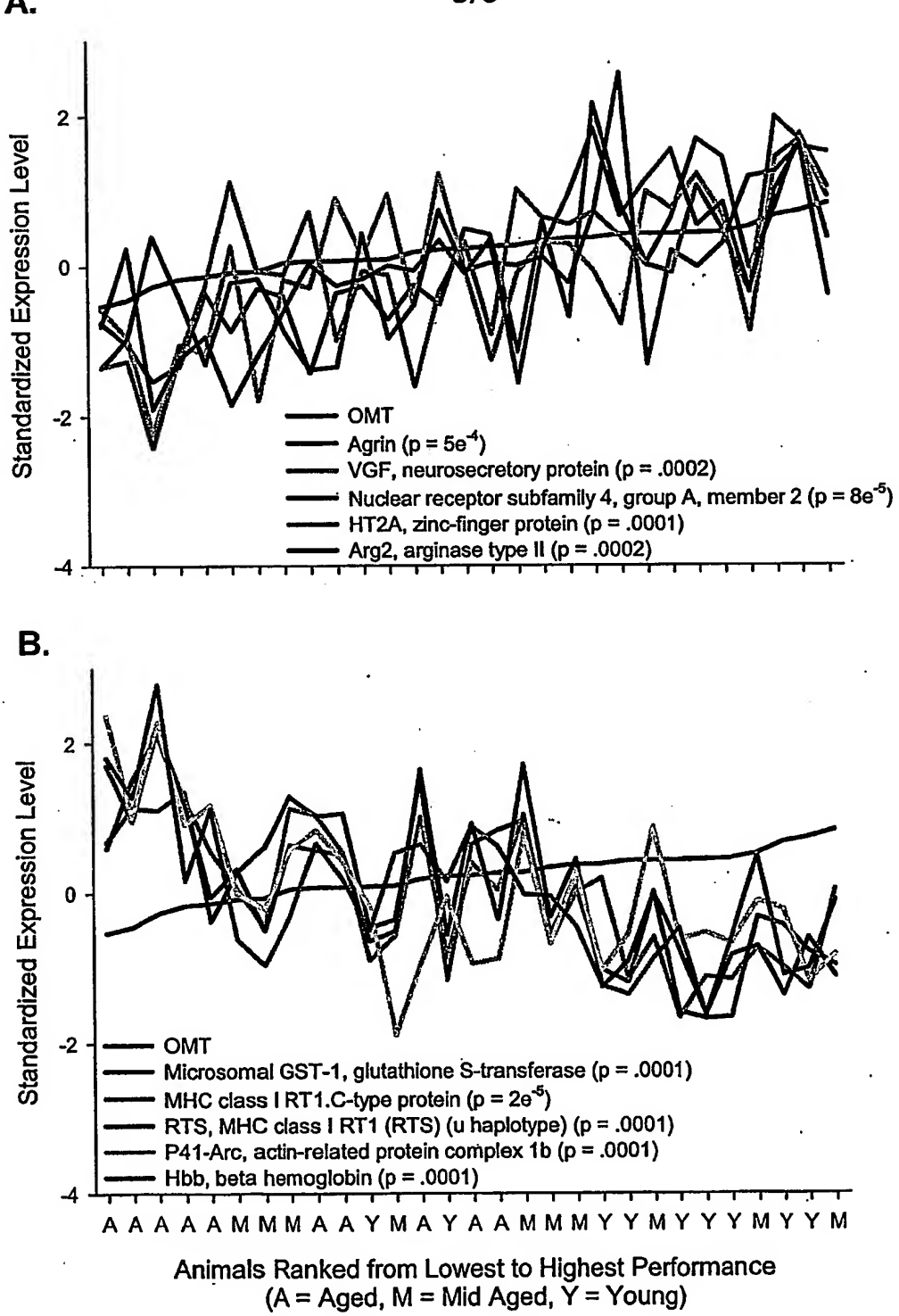
A.
Standardized Expression Level
2
0
-2
-4
OMT
Agrin (p = 5e-4)
VGF, neurosecretory protein (p = .0002)
Nuclear receptor subfamily 4, group A, member 2 (p = 8e-5)
HT2A, zinc-finger protein (p = .0001)
Arg2, arginase type II (p = .0002)
B.
Standardized Expression Level
2
0
-2
-4
OMT
Microsomal GST-1, glutathione S-transferase (p = .0001)
MHC class I RT1.C-type protein (p = 2e-5)
RTS, MHC class I RT1 (RTS) (u haplotype) (p = .0001)
P41-Arc, actin-related protein complex 1b (p = .0001)
Hbb, beta hemoglobin (p = .0001)
A A A A A M M M A A Y M A Y A A M M M Y Y M Y Y Y M Y Y M
Animals Ranked from Lowest to Highest Performance
(A = Aged, M = Mid Aged, Y = Young)

FIG. 3

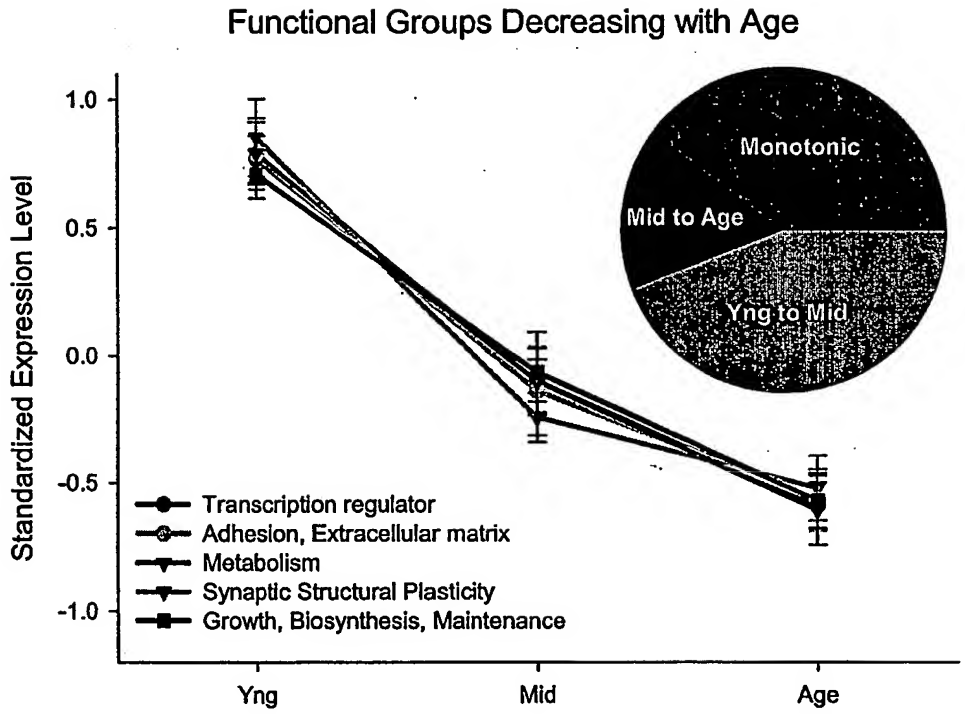
Functional Groups Decreasing with Age
Standardized Expression Level
1.0
0.5
0.0
-0.5
-1.0
Yng
Mid
Age
Monotonic
Mid to Age
Yng to Mid
Transcription regulator
Adhesion, Extracellular matrix
Metabolism
Synaptic Structural Plasticity
Growth, Biosynthesis, Maintenance

FIG. 4

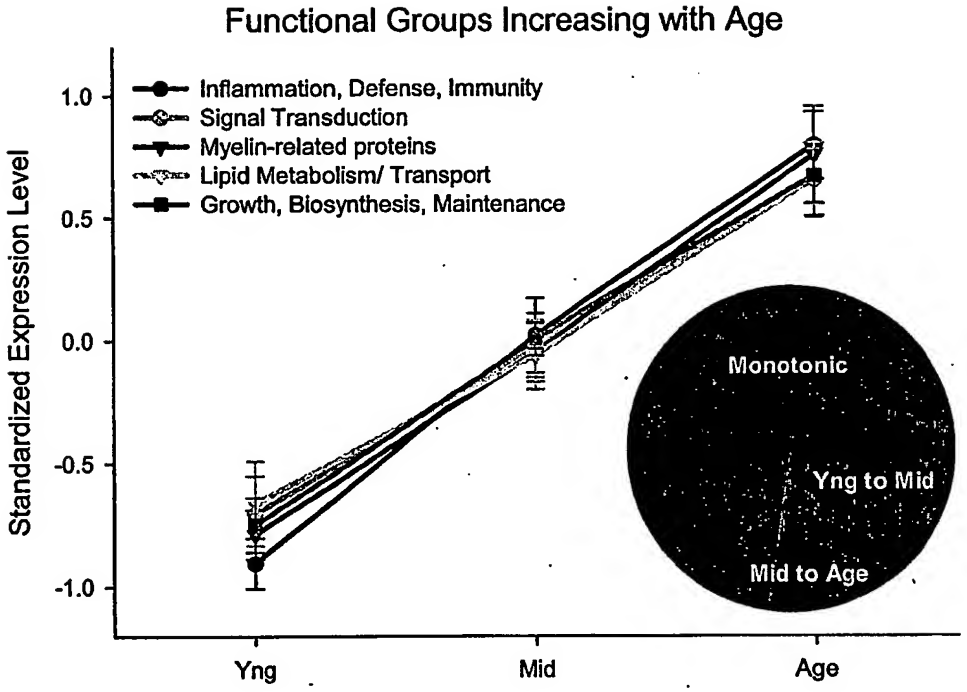
Functional Groups Increasing with Age
Inflammation, Defense, Immunity
Signal Transduction
Myelin-related proteins
Lipid Metabolism/ Transport
Growth, Biosynthesis, Maintenance
Standardized Expression Level
1.0
0.5
0.0
-0.5
-1.0
Yng
Mid
Age
Monotonic
Yng to Mid
Mid to Age

FIG. 5

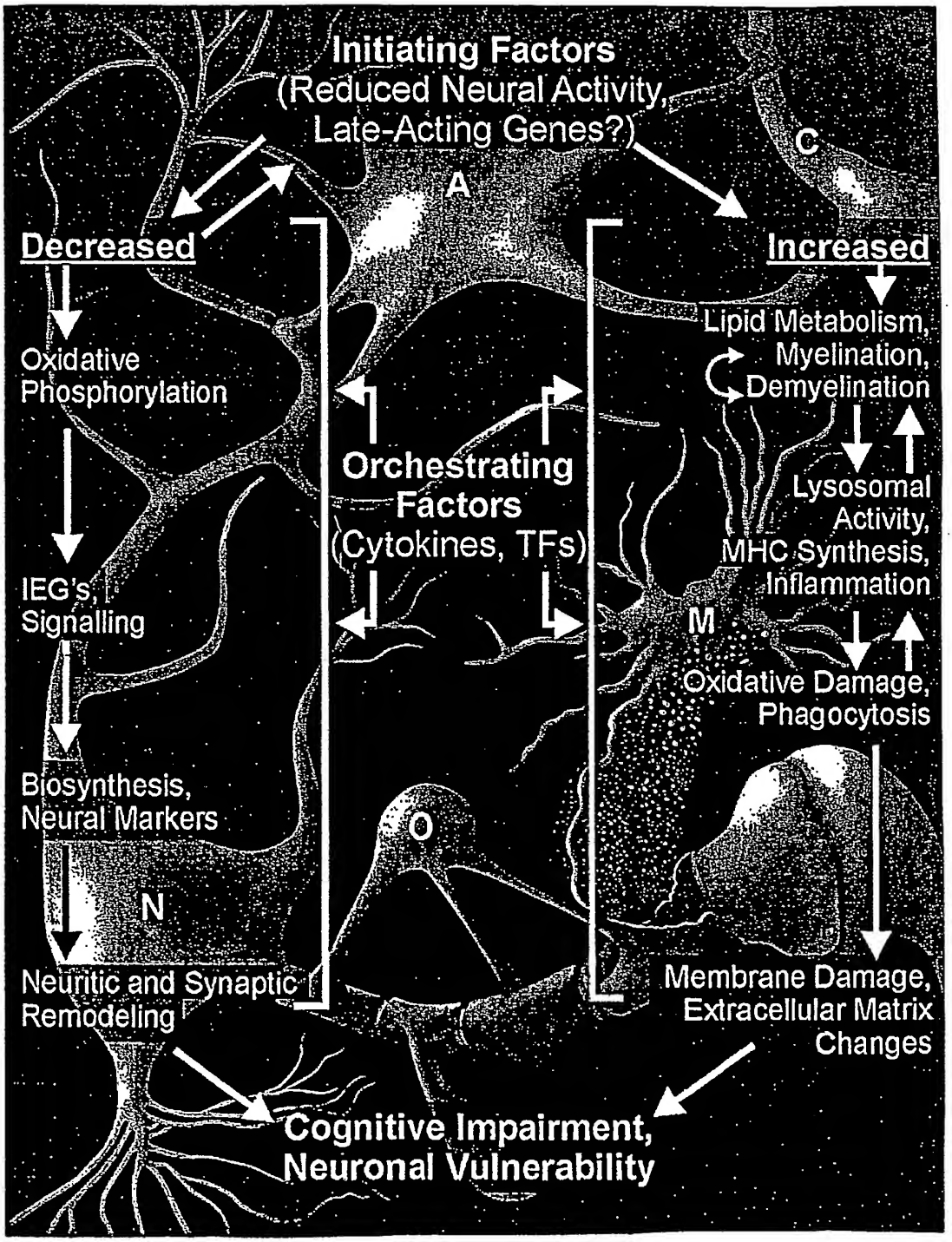
Initiating Factors
(Reduced Neural Activity,
Late-Acting Genes?)
A
C
Decreased
Increased
Oxidative
Phosphorylation
Lipid Metabolism,
Myelination,
Demyelination
Orchestrating
Factors
(Cytokines, TFs)
Lysosomal
Activity,
MHC Synthesis,
Inflammation
IEG's,
Signalling
M
Oxidative Damage,
Phagocytosis
Biosynthesis,
Neural Markers
O
N
Neuritic and Synaptic
Remodeling
Membrane Damage,
Extracellular Matrix
Changes
Cognitive Impairment,
Neuronal Vulnerability

FIG. 6

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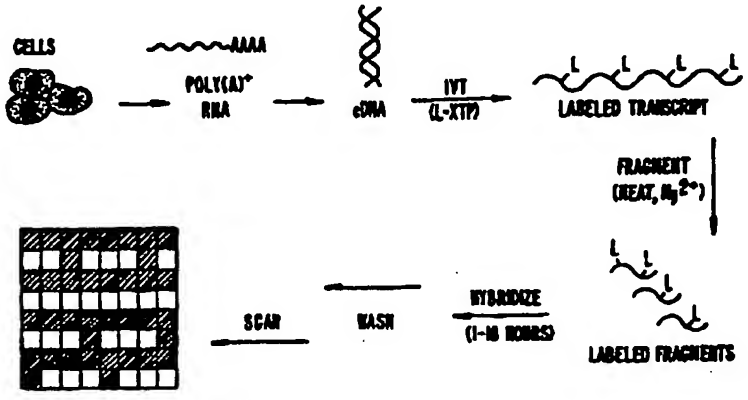

(57) Abstract

This invention provides methods of monitoring the expression levels of a multiplicity of genes. The methods involve hybridizing a nucleic acid sample to a high density array of oligonucleotide probes where the high density array contains oligonucleotide probes complementary to subsequences of target nucleic acids in the nucleic acid sample. In one embodiment, the method involves providing a pool of target nucleic acids comprising RNA transcripts of one or more target genes, or nucleic acids derived from the RNA transcripts, hybridizing said pool of nucleic acids to an array of oligonucleotide probes immobilized on surface, where the array comprising more than 100 different oligonucleotides and each different oligonucleotide is localized in a predetermined region of the surface, the density of the different oligonucleotides is greater than about 60 different oligonucleotides per 1 cm^2, and the oligonucleotide probes are complementary to the RNA transcripts or nucleic acids derived from the RNA transcripts; and quantifying the hybridized nucleic acids in the array.

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EXPRESSION MONITORING BY HYBRIDIZATION TO HIGH DENSITY OLIGONUCLEOTIDE ARRAYS

CROSS REFERENCE TO RELATED APPLICATIONS

This is a continuation-in-part of U.S.S.N. 08/529,115 filed on September 15, 1995 which is herein incorporated by reference for all purposes.

BACKGROUND OF THE INVENTION

Many disease states are characterized by differences in the expression levels of various genes either through changes in the copy number of the genetic DNA or through changes in levels of transcription (*e.g.* through control of initiation, provision of RNA precursors, RNA processing, *etc.*) of particular genes. For example, losses and gains of genetic material play an important role in malignant transformation and progression. These gains and losses are thought to be "driven" by at least two kinds of genes. Oncogenes are positive regulators of tumorgenesis, while tumor suppressor genes are negative regulators of tumorgenesis (Marshall, *Cell*, 64: 313-326 (1991); Weinberg, Science, 254: 1138-1146 (1991)). Therefore, one mechanism of activating unregulated growth is to increase the number of genes coding for oncogene proteins or to increase the level of expression of these oncogenes (*e.g.* in response to cellular or environmental changes), and another is to lose genetic material or to decrease the level of expression of genes that code for tumor suppressors. This model is supported by the losses and gains of genetic material associated with glioma progression (Mikkelson *et al.* J. Cellular Biochm. 46: 3-8 (1991)). Thus, changes in the expression (transcription) levels of

particular genes (*e.g.* oncogenes or tumor suppressors), serve as signposts for the presence and progression of various cancers.

Similarly, control of the cell cycle and cell development, as well as diseases, are characterized by the variations in the transcription levels of particular genes. Thus, for example, a viral infection is often characterized by the elevated expression of genes of the particular virus. For example, outbreaks of *Herpes simplex*, Epstein-Barr virus infections (*e.g.* infectious mononucleosis), cytomegalovirus, Varicella-zoster virus infections, parvovirus infections, human papillomavirus infections, *etc.* are all characterized by elevated expression of various genes present in the respective virus. Detection of elevated expression levels of characteristic viral genes provides an effective diagnostic of the disease state. In particular, viruses such as herpes simplex, enter quiescent states for periods of time only to erupt in brief periods of rapid replication. Detection of expression levels of characteristic viral genes allows detection of such active proliferative (and presumably infective) states.

Oligonucleotide probes have long been used to detect complementary nucleic acid sequences in a nucleic acid of interest (the "target" nucleic acid) and have been used to detect expression of particular genes (*e.g.*, a Northern Blot). In some assay formats, the oligonucleotide probe is tethered, *i.e.*, by covalent attachment, to a solid support, and arrays of oligonucleotide probes immobilized on solid supports have been used to detect specific nucleic acid sequences in a target nucleic acid. *See, e.g.*, PCT patent publication Nos. WO 89/10977 and 89/11548. Others have proposed the use of large numbers of oligonucleotide probes to provide the complete nucleic acid sequence of a target nucleic acid but failed to provide an enabling method for using arrays of immobilized probes for this purpose. *See* U.S. Patent Nos. 5,202,231 and 5,002,867 and PCT patent publication No. WO 93/17126.

The use of "traditional" hybridization protocols for monitoring or quantifying gene expression is problematic. For example two or more gene products of approximately the same molecular weight will prove difficult or impossible to distinguish in a Northern blot because they are not readily separated by electrophoretic methods.

Similarly, as hybridization efficiency and cross-reactivity varies with the particular subsequence (region) of a gene being probed it is difficult to obtain an accurate and reliable measure of gene expression with one, or even a few, probes to the target gene.

The development of VLSIPS™ technology provided methods for synthesizing arrays of many different oligonucleotide probes that occupy a very small surface area. *See* U.S. Patent No. 5,143,854 and PCT patent publication No. WO 90/15070. U.S. Patent application Serial No. 082,937, filed June 25, 1993, describes methods for making arrays of oligonucleotide probes that can be used to provide the complete sequence of a target nucleic acid and to detect the presence of a nucleic acid containing a specific nucleotide sequence.

Prior to the present invention, however, it was unknown that high density oligonucleotide arrays could be used to reliably monitor message levels of a multiplicity of preselected genes in the presence of a large abundance of other (non-target) nucleic acids (*e.g.*, in a cDNA library, DNA reverse transcribed from an mRNA, mRNA used directly or amplified, or polymerized from a DNA template). In addition, the prior art provided no rapid and effective method for identifying a set of oligonucleotide probes that maximize specific hybridization efficacy while minimizing cross-reactivity nor of using hybridization patterns (in particular hybridization patterns of a multiplicity of oligonucleotide probes in which multiple oligonucleotide probes are directed to each target nucleic acid) for quantification of target nucleic acid concentrations.

Summary of the Invention

The present invention is premised, in part, on the discovery that microfabricated arrays of large numbers of different oligonucleotide probes (DNA chips) may effectively be used to not only detect the presence or absence of target nucleic acid sequences, but to quantify the relative abundance of the target sequences in a complex nucleic acid pool. In addition, it was also a surprising discovery that relatively short oligonucleotide probes (*e.g.*, 20 mer) are sufficiently specific to allow quantitation of gene expression in complex mixtures of nucleic acids particularly when provided as in high density oligonucleotide probe arrays.

Prior to this invention it was unknown that hybridization to high density probe arrays would permit small variations in expression levels of a particular gene to be identified and quantified in a complex population of nucleic acids that out number the target nucleic acids by 1,000 fold to 1,000,000 fold or more. It was also unknown that the transcription levels of specific genes can be quantitated in a complex nucleic acid mixture with only a few (*e.g.*, less than 20 or even less than 10) relatively short oligonucleotide probes.

Thus, this invention provides for a method of simultaneously monitoring the expression (*e.g.* detecting and or quantifying the expression) of a multiplicity of genes. The levels of transcription for virtually any number of genes may be determined simultaneously. Typically, at least about 10 genes, preferably at least about 100, more preferably at least about 1000 and most preferably at least about 10,000 different genes are assayed at one time.

The method involves providing a pool of target nucleic acids comprising mRNA transcripts of one or more of said genes, or nucleic acids derived from the mRNA transcripts; hybridizing the pool of nucleic acids to an array of oligonucleotide probes immobilized on a surface, where the array comprises more than 100 different oligonucleotides, each different oligonucleotide is localized in a predetermined region of said surface, each different oligonucleotide is attached to the surface through a single covalent bond, the density of the different oligonucleotides is greater than about 60 different oligonucleotides (where different oligonucleotides refers to oligonucleotides having different sequences) per 1 cm^2, and the oligonucleotide probes are complementary to the mRNA transcripts or nucleic acids derived from the mRNA transcripts; and quantifying the hybridized nucleic acids in the array. The method can additionally include a step of quantifying the hybridization of the target nucleic acids to the array. The quantification preferably provides a measure of the levels of transcription of the genes. In a preferred embodiment, the pool of target nucleic acids is one in which the concentration of the target nucleic acids (mRNA transcripts or nucleic acids derived from the mRNA transcripts) is proportional to the expression levels of genes encoding those target nucleic acids.

In a preferred embodiment, the array of oligonucleotide probes is a high density array comprising greater than about 100, preferably greater than about 1,000 more preferably greater than about 16,000 and most preferably greater than about 65,000 or 250,000 or even 1,000,000 different oligonucleotide probes. Such high density arrays comprise a probe density of generally greater than about 60, more generally greater than about 100, most generally greater than about 600, often greater than about 1000, more often greater than about 5,000, most often greater than about 10,000, preferably greater than about 40,000 more preferably greater than about 100,000, and most preferably greater than about 400,000 different oligonucleotide probes per cm^2 (where different oligonucleotides refers to oligonucleotides having different sequences). The oligonucleotide probes range from about 5 to about 50 nucleotides, preferably from about 5 to about 45 nucleotides, still more preferably from about 10 to about 40 nucleotides and most preferably from about 15 to about 40 nucleotides in length. Particularly preferred arrays contain probes ranging from about 20 to about 25 oligonucleotides in length. The array may comprise more than 10, preferably more than 50, more preferably more than 100, and most preferably more than 1000 oligonucleotide probes specific for each target gene. In a preferred embodiment, the array comprises at least 10 different oligonucleotide probes for each gene. In another preferred embodiment, the array 20 or fewer oligonucleotides complementary each gene. Although a planar array surface is preferred, the array may be fabricated on a surface of virtually any shape or even a multiplicity of surfaces.

The array may further comprise mismatch control probes. Where such mismatch controls are present, the quantifying step may comprise calculating the difference in hybridization signal intensity between each of the oligonucleotide probes and its corresponding mismatch control probe. The quantifying may further comprise calculating the average difference in hybridization signal intensity between each of the oligonucleotide probes and its corresponding mismatch control probe for each gene.

The probes present in the high density array can be oligonucleotide probes selected according to selection and optimization methods described below. Alternatively, non-optimal probes may be included in the array, but the probes used for

quantification (analysis) can be selected according to the optimization methods described below.

Oligonucleotide arrays for the practice of this invention are preferably chemically synthesized by parallel immobilized polymer synthesis methods, more preferably by light directed polymer synthesis methods. Chemically synthesized arrays are advantageous in that probe preparation does not require cloning, a nucleic acid amplification step, or enzymatic synthesis. Indeed, the preparation of the probes does not require handling of any biological materials.

The array includes test probes which are oligonucleotide probes each of which has a sequence that is complementary to a subsequence of one of the genes (or the mRNA or the corresponding antisense cRNA) whose expression is to be detected. In addition, the array can contain normalization controls, mismatch controls and expression level controls as described herein.

In a particularly preferred embodiment, the variation between different copies (within and/or between batches) of each array is less than 20%, more preferably less than about 10%, and most preferably less than about 5% where the variation is measured as the coefficient of variation in hybridization intensity averaged over at least 5 oligonucleotide probes for each gene whose expression the array is to detect.

The pool of nucleic acids may be labeled before, during, or after hybridization, although in a preferred embodiment, the nucleic acids are labeled before hybridization. Fluorescence labels are particularly preferred, more preferably labeling with a single fluorophore, and, where fluorescence labeling is used, quantification of the hybridized nucleic acids is by quantification of fluorescence from the hybridized fluorescently labeled nucleic acid. Such quantification is facilitated by the use of a fluorescence microscope which can be equipped with an automated stage to permit automatic scanning of the array, and which can be equipped with a data acquisition system for the automated measurement recording and subsequent processing of the fluorescence intensity information.

In a preferred embodiment, hybridization is at low stringency (*e.g.* about 20°C to about 50°C, more preferably about 30°C to about 40°C, and most preferably about 37°C and 6X SSPE-T or lower) with at least one wash at higher stringency.

Hybridization may include subsequent washes at progressively increasing stringency until a desired level of hybridization specificity is reached.

Quantification of the hybridization signal can be by any means known to one of skill in the art. However, in a particularly preferred embodiment, quantification is achieved by use of a confocal fluorescence microscope. Data is preferably evaluated by calculating the difference in hybridization signal intensity between each oligonucleotide probe and its corresponding mismatch control probe. It is particularly preferred that this difference be calculated and evaluated for each gene. Particularly preferred analytical methods are provided herein.

The pool of target nucleic acids can be the total polyA$^+$ mRNA isolated from a biological sample, or cDNA made by reverse transcription of the RNA or second strand cDNA or RNA transcribed from the double stranded cDNA intermediate. Alternatively, the pool of target nucleic acids can be treated to reduce the complexity of the sample and thereby reduce the background signal obtained in hybridization. In one approach, a pool of mRNAs, derived from a biological sample, is hybridized with a pool of oligonucleotides comprising the oligonucleotide probes present in the high density array. The pool of hybridized nucleic acids is then treated with RNase A which digests the single stranded regions. The remaining double stranded hybridization complexes are then denatured and the oligonucleotide probes are removed, leaving a pool of mRNAs enhanced for those mRNAs complementary to the oligonucleotide probes in the high density array.

In another approach to background reduction, a pool of mRNAs derived from a biological sample is hybridized with paired target specific oligonucleotides where the paired target specific oligonucleotides are complementary to regions flanking subsequences of the mRNAs complementary to the oligonucleotide probes in the high density array. The pool of hybridized nucleic acids is treated with RNase H which digests the hybridized (double stranded) nucleic acid sequences. The remaining single stranded nucleic acid sequences which have a length about equivalent to the region flanked by the paired target specific oligonucleotides are then isolated (*e.g.* by electrophoresis) and used as the pool of nucleic acids for monitoring gene expression.

Finally, a third approach to background reduction involves eliminating or reducing the representation in the pool of particular preselected target mRNA messages (*e.g.*, messages that are characteristically overexpressed in the sample). This method involves hybridizing an oligonucleotide probe that is complementary to the preselected target mRNA message to the pool of polyA$^+$ mRNAs derived from a biological sample. The oligonucleotide probe hybridizes with the particular preselected polyA$^+$ mRNA (message) to which it is complementary. The pool of hybridized nucleic acids is treated with RNase H which digests the double stranded (hybridized) region thereby separating the message from its polyA$^+$ tail. Isolating or amplifying (*e.g.*, using an oligo dT column) the polyA$^+$ mRNA in the pool then provides a pool having a reduced or no representation of the preselected target mRNA message.

It will be appreciated that the methods of this invention can be used to monitor (detect and/or quantify) the expression of any desired gene of known sequence or subsequence. Moreover, these methods permit monitoring expression of a large number of genes simultaneously and effect significant advantages in reduced labor, cost and time. The simultaneous monitoring of the expression levels of a multiplicity of genes permits effective comparison of relative expression levels and identification of biological conditions characterized by alterations of relative expression levels of various genes. Genes of particular interest for expression monitoring include genes involved in the pathways associated with various pathological conditions (*e.g.*, cancer) and whose expression is thus indicative of the pathological condition. Such genes include, but are not limited to the HER2 (c-erbB-2/neu) proto-oncogene in the case of breast cancer, receptor tyrosine kinases (RTKs) associated with the etiology of a number of tumors including carcinomas of the breast, liver, bladder, pancreas, as well as glioblastomas, sarcomas and squamous carcinomas, and tumor suppressor genes such as the P53 gene and other "marker" genes such as RAS, MSH2, MLH1 and BRCA1. Other genes of particular interest for expression monitoring are genes involved in the immune response (*e.g.*, interleukin genes), as well as genes involved in cell adhesion (*e.g.*, the integrins or selectins) and signal transduction (*e.g.*, tyrosine kinases), *etc.*

In another embodiment, this invention provides a method of identifying genes that are effected by one or more drugs, or conversely, screening a number of

drugs to identify those that have an effect on particular gene(s). This involves providing a pool of target nucleic acids from one or more cells contacted with the drug or drugs and hybridizing that pool to any of the high density oligonucleotide arrays described herein. The expression levels of the genes targeted by the probes in the array are determined and compared to expression levels of genes from "control" cells not exposed to the drug or drugs. The genes that are overexpressed or underexpressed in response to the drug or drugs are identified or conversely the drug or drugs that alter expression of one or more genes are identified.

In still yet another embodiment, this invention provide for a composition comprising any of the high density oligonucleotide arrays disclosed herein where the oligonucleotide probes are specifically hybridized to one or more fluorescently labeled nucleic acids (which are the transcription products of genes or derived from those transcription products) thereby forming a fluorescent array in which the fluorescence of the array is indicative of the transcription levels of the multiplicity of genes. One of skill will appreciate that such a hybridized array may be used as a reference, control, or standard (*e.g.*, provided in a kit) or may itself be a diagnostic array indicating the expression levels of a multiplicity of genes in a sample.

This invention also provides kits for simultaneously monitoring expression levels of a multiplicity of genes. The kits include an array of immobilized oligonucleotide probes complementary to subsequences of the multiplicity of target genes, as described herein. The kit may also include instructions describing the use of the array for detection and/or quantification of expression levels of the multiplicity of genes. The kit may additionally include one or more of the following: buffers, hybridization mix, wash and read solutions, labels, labeling reagents (enzymes *etc.*), "control" nucleic acids, software for probe selection, array reading or data analysis and any of the other materials or reagents described herein for the practice of the claimed methods.

In another embodiment, this invention provides for a method of selecting a set of oligonucleotide probes, that specifically bind to a target nucleic acid (*e.g.*, a gene or genes whose expression is to be monitored or nucleic acids derived from the gene or its transcribed mRNA). The method involves providing a high density array of

oligonucleotide probes where the array comprises a multiplicity of probes wherein each probe is complementary to a subsequence of the target nucleic acid. The target nucleic acid is then hybridized to the array of oligonucleotide probes to identify and select those probes where the difference in hybridization signal intensity between each probe and its mismatch control is detectable (preferably greater than about 10% of the background signal intensity, more preferably greater than about 20% of the background signal intensity and most preferably greater than about 50% of the background signal intensity). The method can further comprise hybridizing the array to a second pool of nucleic acids comprising nucleic acids other than the target nucleic acids; and identifying and selecting probes having the lowest hybridization signal and where both the probe and its mismatch control have a hybridization intensity equal to or less than about 5 times the background signal intensity, preferably equal to or less than about 2 times the background signal intensity, more preferably equal to or less than about 1 times the background signal intensity, and most preferably equal or less than about half the background signal intensity.

In a preferred embodiment, the multiplicity of probes can include every different probe of length n that is complementary to a subsequence of the target nucleic acid. The probes can range from about 10 to about 50 nucleotides in length. The array is preferably a high density array as described above. Similarly, the hybridization methods, conditions, times, fluid volumes, detection methods are as herein .

In another embodiment, the invention provides a computer-implemented method of monitoring expression of genes comprising the steps of: receiving input of hybridization intensities for a plurality of nucleic acid probes including pairs of perfect match probes and mismatch probes, the hybridization intensities indicating hybridization affinity between the plurality of nucleic acid probes and nucleic acids corresponding to a gene, and each pair including a perfect match probe that is perfectly complementary to a portion of the nucleic acids and a mismatch probe that differs from the perfect match probe by at least one nucleotide; comparing the hybridization intensities of the perfect match and mismatch probes of each pair; and indicating expression of the gene according to results of the comparing step. Preferably, the differences between the

hybridization intensities of the perfect match and mismatch probes of each pair are calculated.

Additionally, the invention provides a computer-implemented method for monitoring expression of genes comprising the steps of: receiving input of a nucleic acid sequence constituting a gene; generating a set of probes that are perfectly complementary to the gene; and identifying a subset of probes, including less than all of the probes in the set, for monitoring the expression of the gene. Each probe of the set may be analyzed by criteria that specify characteristics indicative of low hybridization or high cross hybridization. The criteria may include if occurrences of a specific nucleotide in a probe crosses a threshold value, if the number of a specific nucleotide that repeats sequentially in a probe crosses a threshold value, if the length of a palindrome in a probe crosses a threshold value, and the like.

Definitions.

The phrase "massively parallel screening" refers to the simultaneous screening of at least about 100, preferably about 1000, more preferably about 10,000 and most preferably about 1,000,000 different nucleic acid hybridizations.

The terms "nucleic acid" or "nucleic acid molecule" refer to a deoxyribonucleotide or ribonucleotide polymer in either single-or double-stranded form, and unless otherwise limited, would encompass known analogs of natural nucleotides that can function in a similar manner as naturally occurring nucleotides.

An oligonucleotide is a single-stranded nucleic acid ranging in length from 2 to about 500 bases.

As used herein a "probe" is defined as an oligonucleotide capable of binding to a target nucleic acid of complementary sequence through one or more types of chemical bonds, usually through complementary base pairing, usually through hydrogen bond formation. As used herein, an oligonucleotide probe may include natural (*i.e.* A, G, C, or T) or modified bases (7-deazaguanosine, inosine, *etc.*). In addition, the bases in oligonucleotide probe may be joined by a linkage other than a phosphodiester bond, so long as it does not interfere with hybridization. Thus, oligonucleotide probes may be

peptide nucleic acids in which the constituent bases are joined by peptide bonds rather than phosphodiester linkages.

The term "target nucleic acid" refers to a nucleic acid (often derived from a biological sample), to which the oligonucleotide probe is designed to specifically hybridize. It is either the presence or absence of the target nucleic acid that is to be detected, or the amount of the target nucleic acid that is to be quantified. The target nucleic acid has a sequence that is complementary to the nucleic acid sequence of the corresponding probe directed to the target. The term target nucleic acid may refer to the specific subsequence of a larger nucleic acid to which the probe is directed or to the overall sequence (*e.g.*, gene or mRNA) whose expression level it is desired to detect. The difference in usage will be apparent from context.

"Subsequence" refers to a sequence of nucleic acids that comprise a part of a longer sequence of nucleic acids.

The term "complexity"is used here according to standard meaning of this term as established by Britten *et al. Methods of Enzymol.* 29:363 (1974). See, also *Cantor and Schimmel Biophysical Chemistry: Part III* at 1228-1230 for further explanation of nucleic acid complexity.

"Bind(s) substantially" refers to complementary hybridization between a probe nucleic acid and a target nucleic acid and embraces minor mismatches that can be accommodated by reducing the stringency of the hybridization media to achieve the desired detection of the target polynucleotide sequence.

The phrase "hybridizing specifically to", refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent conditions when that sequence is present in a complex mixture (*e.g.*, total cellular) DNA or RNA. The term "stringent conditions" refers to conditions under which a probe will hybridize to its target subsequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH, and nucleic acid concentration) at which 50% of the probes

complementary to the target sequence hybridize to the target sequence at equilibrium. (As the target sequences are generally present in excess, at Tm, 50% of the probes are occupied at equilibrium). Typically, stringent conditions will be those in which the salt concentration is at least about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (*e.g.*, 10 to 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide.

The term "perfect match probe" refers to a probe that has a sequence that is perfectly complementary to a particular target sequence. The test probe is typically perfectly complementary to a portion (subsequence) of the target sequence. The perfect match (PM) probe can be a "test probe", a "normalization control" probe, an expression level control probe and the like. A perfect match control or perfect match probe is, however, distinguished from a "mismatch control" or "mismatch probe."

The term "mismatch control" or "mismatch probe" refer to probes whose sequence is deliberately selected not to be perfectly complementary to a particular target sequence. For each mismatch (MM) control in a high-density array there typically exists a corresponding perfect match (PM) probe that is perfectly complementary to the same particular target sequence. The mismatch may comprise one or more bases. While the mismatch(s) may be locates anywhere in the mismatch probe, terminal mismatches are less desirable as a terminal mismatch is less likely to prevent hybridization of the target sequence. In a particularly preferred embodiment, the mismatch is located at or near the center of the probe such that the mismatch is most likely to destabilize the duplex with the target sequence under the test hybridization conditions.

The terms "background" or "background signal intensity" refer to hybridization signals resulting from non-specific binding, or other interactions, between the labeled target nucleic acids and components of the oligonucleotide array (*e.g.*, the oligonucleotide probes, control probes, the array substrate, *etc.*). Background signals may also be produced by intrinsic fluorescence of the array components themselves. A single background signal can be calculated for the entire array, or a different background signal may be calculated for each target nucleic acid. In a preferred embodiment, background is calculated as the average hybridization signal intensity for the lowest 5%

to 10% of the probes in the array, or, where a different background signal is calculated for each target gene, for the lowest 5% to 10% of the probes for each gene. Of course, one of skill in the art will appreciate that where the probes to a particular gene hybridize well and thus appear to be specifically binding to a target sequence, they should not be used in a background signal calculation. Alternatively, background may be calculated as the average hybridization signal intensity produced by hybridization to probes that are not complementary to any sequence found in the sample (*e.g.* probes directed to nucleic acids of the opposite sense or to genes not found in the sample such as bacterial genes where the sample is mammalian nucleic acids). Background can also be calculated as the average signal intensity produced by regions of the array that lack any probes at all.

The term "quantifying" when used in the context of quantifying transcription levels of a gene can refer to absolute or to relative quantification. Absolute quantification may be accomplished by inclusion of known concentration(s) of one or more target nucleic acids (*e.g.* control nucleic acids such as Bio B or with known amounts the target nucleic acids themselves) and referencing the hybridization intensity of unknowns with the known target nucleic acids (*e.g.* through generation of a standard curve). Alternatively, relative quantification can be accomplished by comparison of hybridization signals between two or more genes, or between two or more treatments to quantify the changes in hybridization intensity and, by implication, transcription level.

The"percentage of sequence identity" or "sequence identity" is determined by comparing two optimally aligned sequences or subsequences over a comparison window or span, wherein the portion of the polynucleotide sequence in the comparison window may optionally comprise additions or deletions (*i.e.*, gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical subunit (*e.g.* nucleic acid base or amino acid residue) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Percentage sequence identity when calculated using the programs GAP or BESTFIT (see below) is calculated using default gap weights.

Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman, *Adv. Appl. Math.* 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch *J. Mol. Biol.* 48: 443 (1970), by the search for similarity method of Pearson and Lipman, *Proc. Natl. Acad. Sci. USA* 85: 2444 (1988), by computerized implementations of these algorithms (including, but not limited to CLUSTAL in the PC/Gene program by Intelligenetics, Moutain View, California, GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, Wisconsin, USA), or by inspection. In particular, methods for aligning sequences using the CLUSTAL program are well described by Higgins and Sharp in *Gene*, 73: 237-244 (1988) and in *CABIOS* 5: 151-153 (1989)).

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a schematic of expression monitoring using oligonucleotide arrays. Extracted poly $(A)^+$ RNA is converted to cDNA, which is then transcribed in the presence of labeled ribonucleotide triphosphates. L is either biotin or a dye such as fluorescein. RNA is fragmented with heat in the presence of magnesium ions. Hybridizations are carried out in a flow cell that contains the two-dimensional DNA probe arrays. Following a brief washing step to remove unhybridized RNA, the arrays are scanned using a scanning confocal microscope. Alternatives in which cellular mRNA is directly labeled without a cDNA intermediate are described in the Examples. Image analysis software converts the scanned array images into text files in which the observed intensities at specific physical locations are associated with particular probe sequences.

Fig. 2A shows a fluorescent image of a high density array containing over 16,000 different oligonucleotide probes. The image was obtained following hybridization (15 hours at 40°C) of biotin-labeled randomly fragmented sense RNA transcribed from the murine B cell (Tl0) cDNA library, and spiked at the level of 1:3,000 (50 pM equivalent to about 100 copies per cell) with 13 specific RNA targets. The brightness at any location is indicative of the amount of labeled RNA hybridized to the particular oligonucleotide probe. Fig. 2B shows a small portion of the array (the boxed region of Fig. 2A) containing probes

for IL-2 and IL-3 RNAS, For comparison, Fig. 2C shows shown the same region of the array following hybridization with an unspiked T10 RNA samples (T10 cells do not express IL-2 and IL-3). The variation in the signal intensity was highly reproducible and reflected the sequence dependence of the hybridization efficiencies. The central cross and the four corners of the array contain a control sequence that is complementary to a biotin-labeled oligonucleotide that was added to the hybridization solution at a constant concentration (50 pM). The sharpness of the images near the boundaries of the features was limited by the resolution of the reading device (11.25 μm) and not by the spatial resolution of the array synthesis. The pixels in the border regions of each synthesis feature were systematically ignored in the quantitative analysis of the images.

Fig. 3 provides a log/log plot of the hybridization intensity (average of the PM-MM intensity differences for each gene) versus concentration for 11 different RNA targets. The hybridization signals were quantitatively related to target concentration. The experiments were performed as described in the Examples herein and in Fig. 2. The ten 10 cytokine RNAs (plus *bioB*) were spiked into labeled T10 RNA at levels ranging from 1:300,000 to 1:3,000. The signals continued to increase with increased concentration up to frequencies of 1:300, but the response became sublinear at the high levels due to saturation of the probe sites, The linear range can be extended to higher concentrations by using shorter hybridization times. RNAs from genes expressed in T10 cells (IL-10, β-actin and GAPDH) were also detected at levels consistent with results obtained by probing cDNA libraries.

Fig. 4 shows cytokine mRNA levels in the murine 2D6 T helper cell line at different times following stimulation with PMA and a calcium ionophore. Poly (A)$^+$ RNA was extracted at 0, 2, 6, and 24 hours following stimulation and converted to double stranded cDNA containing an RNA polymerase promoter. The cDNA pool was then transcribed in the presence of biotin labeled ribonucleotide triphosphates, fragmented, and hybridized to the oligonucleotide probe arrays for 2 and 22 hours. The fluorescence intensities were converted to RNA frequencies by comparison with the signals obtained for a bacterial RNA (biotin synthetase) spiked into the samples at known amounts prior to hybridization. A signal of 50,000 corresponds to a frequency of approximately 1:100,000 to a frequency of 1:5,000, and a signal of 100 to a frequency of 1:50,000. RNAs for IL-2,

IL-4, IL-6, and IL-12p40 were not detected above the level of approximately 1:200,000 in these experiments. The error bars reflect the estimated uncertainty (25 percent) in the level for a given RNA relative to the level for the same RNA at a different time point. The relative uncertainty estimate was based on the results of repeated spiking experiments, and on repeated measurements of IL-10, β-actin and GAPDH RNAs in preparations from both T10 and 2D6 cells (unstimulated). The uncertainty in the absolute frequencies includes message-to-message differences in the hybridization efficiency as well as differences in the mRNA isolation, cDNA synthesis, and RNA synthesis and labeling steps. The uncertainty in the absolute frequencies is estimated to be a factor of three.

Fig. 5 shows a fluorescence image of an array containing over 63,000 different oligonucleotide probes for 118 genes. The image was obtained following overnight hybridization of a labeled murine B cell RNA sample. Each square synthesis region is 50 x 50 μm and contains 107 to 108 copies of a specific oligonucleotide. The array was scanned at a resolution of 7.5 μm in approximately 15 minutes. The bright rows indicate RNAs present at high levels. Lower level RNAs were unambiguously detected based on quantitative evaluation of the hybridization patterns. A total of 21 murine RNAs were detected at levels ranging from approximately 1:300,000 to 1:100. The cross in the center, the checkerboard in the corners, and the MUR-1 region at the top contain probes complementary to a labeled control oligonucleotide that was added to all samples.

Fig. 6 shows an example of a computer system used to execute the software of an embodiment of the present invention.

Fig. 7 shows a system block diagram of a typical computer system used to execute the software of an embodiment of the present invention.

Fig. 8 shows the high level flow of a process of monitoring the expression of a gene by comparing hybridization intensities of pairs of perfect match and mismatch probes.

Fig. 9 shows the flow of a process of determining if a gene is expressed utilizing a decision matrix.

Figs. 10A and 10B show the flow of a process of determining the expression of a gene by comparing baseline scan data and experimental scan data.